STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE EWE.

by

RONALD GRANT, B.Sc.

(Ministry of Agriculture
Research Scholar in Agricultural Science.)

T H E S I S

PRESENTED TO THE FACULTY OF SCIENCE

of the

UNIVERSITY OF EDINBURGH

In fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

by

RONALD GRANT, B.Sc.

from the

INSTITUTE OF ANIMAL GENETICS.

April 1933.

TABLE OF CONTENTS.

I : INTRODUCTION.

Knowledge of the reproductive mechanisms of the mammals has advanced with remarkable rapidity since Stockard and Papanicolaou (158) in 1917, described in detail the cyclical changes in the character of the vaginal mucosa associated with the oestrous cycle in the guinea-pig, to which Retterer (132) and Latase (90) drew attention more than twenty years previously. The vaginal smear technique elaborated by Stockard and Papanicolaou has been proved to be a most useful weapon for attacking the problems of sex-physiology of the smaller mammals and has been applied at one time or another to almost every species of mammal of which the reproductive phenomena have been investigated.

Nearly all the investigators in this field have directed their attention to the small "laboratory" mammals, however, and it is to these almost exclusively that methods of experimental inter-:ference have been applied. It is debatable whether far more light might not be thrown upon the physiological mechanisms of reproduction in mammals by the comparative study of the normal reproductive processes in many different species than by the detailed experimental study of a few forms; and quite certainly it is not possible to apply, without question, the theoretical conceptions elaborated to explain the reproductive mechanisms of one highly specialised group, such as the rodents, to the whole group/

group of mammals, as many authors have been prone to do.

Practically nothing is yet known of the reproductive processes in the great group of Ungulates except in so far as the domesticated animals belonging to this group have been studied. The normal reproductive phenomena in the cow have been the subject of an extensive study by Hammond (66) while Frei and his co-workers have extended Hammond's work, particularly in its relation to sterile animals. Corner (44) and McKenzie (111) have studied certain aspects of the reproductive processes in the sow. Marshall (101) and Assheton (11) have given short accounts of certain of the reproductive phenomena in the ewe. Minor papers dealing with the goat and mare have also been pub-:lished but no concise accounts are yet available for these forms.

When the present investigation was commenc-:ed Marshall's paper was the only published account of the sex-physiological processes in the sheep, although a number of papers dealing with the problem of fertility in the ewe and a number of minor papers, such as that by Küpfer (86) dealing with ovarian changes, had appeared. Schmaltz (144) in his comparative account of the sexual processes of the domestic mammals, in dealing with the sheep was able to do little more than state the problems which remained/

remained to be solved. More recently, however, a number of short studies on the sheep have been published. Quinlan and Maré (130) have described the gross ovarian changes in the South African Merino ewe and have determined the duration and periodicity of oestrum in that breed of sheep. McKenzie and Phillips (112) have studied oestrous periodicity in Southdown, Shropshire and Hampshire sheep. Cole and Miller (40, 42) and Darlow and Hawkins (47) have studied the changes in the vaginal fluid and Casida and McKenzie (37) have given a brief account of the structural changes in the generative tract. None of these accounts can be considered to be complete or final even in the very limited fields which they are intended to cover.

The investigation of which this Thesis forms a report was commenced with the intention of providing a detailed survey of reproductive phenomena in the ewe which might be comparable with Hammond's monograph on the physiology of reproduction in the cow. This objective has, as yet, been only partial-:ly attained. It has been found that the structural changes in the reproductive organs are subject to a very great amount of individual variation and the accurate determination of the scope of these variations has necessitated the study of far more material than was at first anticipated. The present account/

account covers the principal changes in the non-pregnant animal. A brief account of the changes in the pregnant animal has been given in order that the structural modifications of the reproductive tract which take place during the dioestrous cycle may be given a functional interpretation.

It is hoped that this study may ultimately form the basis for further experimental investigation of reproductive processes in the ewe, particularly in relation to breeding problems encountered in practical sheep husbandry; and that at the same time it may lay the foundations for a more comprehensive understanding of the normal mechanism controlling reproductive phenomena in ruminants generally.

In approaching this subject the writer endeavoured to adopt the attitude that an entirely new field of study was being entered, that each detail required careful investigation on its own merits and that it was not sufficient simply to com-:pare the phenomena in the sheep with those which have been described in other mammals. It is in-:evitable, therefore, that certain aspects of the subject must, at first sight, appear to have been treated in unnecessary detail, but it is hoped that in this way the drawing of unwarrantable parallels between the phenomena displayed by the ewe and those displayed by other animals may have been avoided.

That/

That this attitude has been justified is shown by many of the results which have emerged: for example, it has been shown that the wave of mitotic activity which passes over the uterine epithelium during metoestrum, and which might readily have been interpreted, on the basis of conditions in the uterus of the rabbit for instance, during the luteal phase, as due to the action of a hormone produced by the corpus luteum, cannot be due to the presence of a luteal hormone since mitotic activity commences before the luteal cells have been formed. Less detailed investigation would have led to the conclusion that mitotic activity in the uterine epithelium occurred under the influence of a hormone of the corpus luteum.

The progress of this study has nevertheless been guided throughout by reference to Schmaltz's monograph on the sexual life of the domestic animals in general and to Hammond's monograph on reproduction in the cow. Constant reference to these two works has probably prevented many errors of interpretation which would otherwise have been made.

I : MATERIAL AND METHODS.

1. MATERIAL.

The material for this investigation was derived from a number of separate sources and all observations made have thus been subject to adequate control. The sources utilised were -

(1) A group of 17 experimental ewes, particulars of which are recorded in Appendix Table 3. From these ewes vaginal smears were taken daily or more frequent-:ly, from the beginning of September 1931 until mid-May of the following year. This period covered the whole of the breeding season and in most cases a considerable period of anoestrum at each end of the breeding period. Seven of these ewes were killed at various phases of the oestrous cycle (see Appendix Table 1) and their genitalia subjected to detailed histological examination. Two others were served by a fertile ram at the 6th oestrous period in each case, and the vaginal changes followed through pregnancy, smears being taken daily at first and less frequently later on. Smears were also taken daily from these two ewes during the first month of lactation. Smears were taken from the remainder of the group at short intervals throughout the summer of 1932.

A vasectomised ram was used throughout, with this and with the other groups of ewes kept at the Institute, to determine the onset and duration of sexual desire. This ram was used also with the ewes employed during the 1932-33 season. A vasectomised ram is probably a great deal more satisfactory for detecting oestrus than "aproned" rams such as have been used by other workers, since the ligation of the vasa diferentia seems to prevent, or greatly mitigate, the sexual exhaustion that usually follows excessive use of a normal ram. This particular ram showed no sign of failing sexual activity throughout the time that he was used, although, during the first season, he accomplished about four to five hundred "services". Vasectomised and vasoligated rams appear to gain considerably in libido (See also Humphrey (74)). The present ram would serve (and apparently ejaculation occurred at each service) five or six ewes in succession within a quarter of an hour. If left with a ewe throughout her heat period copulation seemed to be repeated about every half hour.

During the whole of the breeding season the vasectomised ram was "keeled" (i.e., coloured chalk mixed with whale oil was rubbed on his abdomen immediately in front of the penis, so that he would mark any ewes that were served) and run with the ewes; the flock was examined for ewes on heat at intervals

of/

of eight hours or less. During the oestrous period itself the ewes in heat were generally separated from the main flock and from the ram, the progress of oestrum being observed either by "hand-serving" with the vasectomised ram or by observing the ewe's behaviour when separated from the ram by a fence only. When it was desired that a ewe should not be served by the ram during an oestrous period she was separated from the flock on the 14th day following the onset of the previous heat period and the onset of heat determined by the behaviour of the ewe towards the ram while separated from him by a fence.

No smears were recorded as "normal" which were taken within eight hours of service. Actually, the effect of the male secretion seemed to disappear about 3 hours after copulation since during oestrum the vaginal contents are continually changing, owing to expulsion of material from the vulva.

(2) The entire 1931 breeding stock of ewes at the Institute (89 ewes) was utilised as a direct control for the experimental animals. Vaginal smears were taken from these ewes on two occasions during the breeding season, once early in the season (November the 2nd) and again as each lot of ewes was put with the fertile ram with which it was to be bred. After the first smearing all the ewes were put with the vasectomised ram in order to determine which ewes were

on/

on heat at the time. After the second smearing all the ewes went to the fertile rams and, from the service dates of the ewes, the stage of the oestrous cycle at the time of smearing determined approximate-:ly. For example, a ewe served 10 days after the smearing would have been at about the 6th day of the previous cycle when smeared, since the complete cycle lasts 16-17 days.

(3) During the autumn of 1931, two, and during the autumn of 1932, twenty ewes, not of the experimental group, were slaughtered at the Institute. Eight of these had not yet come into their breeding season at the time of slaughter, 5 were killed at various stages of the oestrous cycle, and 8 were killed during the first month of pregnancy. One other ewe was killed in March, 7 days after parturition. (See Appendix Table 1.)

(4) Slaughter House Material. From January 1931 until June 1932 the Edinburgh Abattoir was visited weekly (less frequently during the mid-summer) and genitalia from freshly killed ewes examined. Thirty to fifty sets of genitalia were examined each week so that the data collected represent about 2,000 ewes. All this material was classified according to the state of the ovaries (see page 136). At the same time data regarding the general appearance of the generative/

generative tract (oedema, vascularity, etc.) and macroscopic characters of the Fallopian tubes, uterus, cervix, vagina and vaginal secretions, were recorded in tabular form for each ewe. In addition about 50 reproductive tracts from ewes killed earlier in the day were examined roughly for anatomical abnormalities or aberrant conditions of the ovaries.

From a total of 352 of these ewes, tissues were taken for histological examination. The routine areas so examined were the ovaries, mid-Fallopian tube, mid-uterus, mid-cervix and mid-ventral vagina. In most cases, however, several other areas were also examined microscopically. All tissues were put into fixative at the abattoir. Vaginal smears were taken from all ewes from which tissues were taken and also from many others. Many uterine smears were also made.

Most of the sheep killed at the Edinburgh abattoir come from lowland and hill farms in the south-eastern counties of Scotland, but there is always a considerable number from other parts of Scotland, including the Highland mountain farms and those in the extreme north. The only district not, as a rule, well represented is the south-west. Thus the material examined may quite reasonably be regard-:ed as representative of Scotland as a whole. During certain seasons, however, sheep from particular localities predominated: Highland sheep were most common/

common in winter and early spring. Most of the sheep coming from hill or mountain farms were either Cheviots or Blackfaces: those coming from the low ground farms were mainly crossbreds involving, in many cases, a considerable number of breeds. The ewes were divisible roughly into three age groups, i) lambs 5-9 months old, ii) "Gimmers" about 18 months old, and iii) ewes 3 or more years old which had raised one or more crops of lambs: many of these were pregnant when killed. The approximate age and (where possible) breed of each ewe were recorded.

(5) In the autumn of 1932 a group of 28 ewes was employed in a preliminary experiment which aimed at the experimental elucidation of the factors controll-:ing the onset of the breeding season. The consti-:tution of this group and the experimental procedure adopted with them are set out elsewhere (pages 70-77.). The 1932 breeding stock was used as a control to this experiment, and at the same time provided data regarding breed differences in the time of commencement of the breeding season.

2. TECHNICAL PROCEDURE EMPLOYED IN THE TAKING AND EXAMINATION OF VAGINAL SAMPLES.

For the removal of samples of the vaginal fluid/

fluid from the experimental ewes a glass rod 20 centimetres long with a flattened, spatulate end, 1 centimetre in diameter and inclined to the rod at an angle of 45 degrees, was employed. A speculum of the narrow nasal type was used to dilate the vulva: the advantage of this type lay in the fact that it allowed practically the whole surface of the vestibule as well as that of the vagina to be sampled.

The lips of the vulva and the surrounding area of the skin were cleansed thoroughly immediately before smearing with a swab of cotton wool which had been soaked in 2½% Liquor cresolis saponatus solution. The speculum was then inserted into the vestibule and dilated. It was found advisable to moisten the speculum with soap solution before insertion as the lips of the vulva are usually rather dry and, if the speculum were inserted dry, there was risk of injuring the vestibular mucous membrane. The smearing rod was inserted dry and was passed right into the vagina until the spade end came in contact with the external os of the cervix. The rod was then withdrawn slightly and the area about 2 centi-:metres posterior to the cervix scraped gently with the spade, turning the rod meanwhile so that the spade came in contact with the whole circumference of the vagina. In this way a fairly representative sample/

sample of the contents of all parts of the vagina was obtained, for the stem of the rod carried away material from the posterior parts of the vagina, while the fact that the spade was set at an angle enabled the smear from the more remote parts to be withdrawn without contamination. Material from several parts of the rod was transferred directly to a glass slip and spread by that part of the rod which had not been inserted into the vagina. Before use on another ewe the speculum and rod were sterilised in 2½% Cresol soap solution and wiped clean.

Several other techniques were tested but none of these was found to be so satisfactory as that which has been outlined. The swab, and the wire loop methods were found to be totally inadequate since the samples removed by these instruments were representative of only a very small area of the vagina. Washing out with saline required the use of such a large volume of fluid that the use of this method was impracticable. No doubt, had this method been employed, some of the effects referable to the mechanical irritation caused by smearing might have been avoided. Darlow and Hawkins (47) used the more complicated method, first employed by McKenzie, in which a glass rod sliding within an open glass tube on the trocar and canula principle is used, but this method was found to be less satisfactory than the/

the simple rod.

It was ascertained by post-mortem examination of experimental ewes that the method described above gave a remarkably good picture of the character of the surface layers of the vaginal epithelium. Many smears were taken also from the vestibule alone, for comparative purposes: for these the same instruments were used.

Absolute methyl alcohol, free from acetone, was employed as a fixative for all smears. No difference in the quality of fixation was observable between smears fixed while still wet and those fixed after drying, but the latter adhered to the slide somewhat better and drying of the smear before fixation was adopted as a regular technique. As a standard staining method for smears the Giemsa technique was adopted. This stain, in common with all the Romanowsky stains, is a complex mixture of dyes (mainly methylene blue, eosinates of methylene blue, methylene azure and methylene violet) and different batches are liable to considerable variation in composition and therefore in staining properties. It is also very sensitive to variations in hydrogen ion concentration. For these reasons the same bulk solution, made up from dry stains at the commencement of the experiments, was used throughout, and a strictly standardised staining method was employed./

employed. The stock solution was diluted for use with fresh distilled water and the time of staining was uniformly eighteen minutes. Differentiation was carried out in fresh distilled water. The smears were then blotted dry and kept unmounted except in a few instances, in which Dammar was employed as a mounting medium. It was found that, provided the glass slides used were perfectly clean, a uniform staining reaction could be obtained and smears pre-:pared in this way kept excellently. All the slides used were steeped in a mixture of equal parts of saturated potassium bichromate solution and concentrated sulphuric acid for a week, washed in running water for 24 hours, rinsed in distilled water and stored in absolute alcohol.

The use of the McJunkin-Haden buffer (pH 6.4) as a diluting fluid for the stain was tried, but this possessed no advantage over fresh distilled water. Several other stains were used occasionally, particularly Delafield's haematoxylin and eosin; Ehrlich's haematoxylin and eosin; Heidenhein's iron haematoxylin; Gram's bacteriological stain; Methylene blue; and Mucicarmine. None of these gave the same degree of differentiation that was obtained with Giemsa. Exactly the same fixation and staining techniques were employed for the smears obtained post-mortem at the slaughter houses.

When vaginal smears were being taken observations/

observations were made on certain other conditions of the reproductive organs which might be subject to cyclical variation. The chief of these were:-

i) Changes in the condition of the external genital organs, i.e., of the vulva. These observations concerned the degree of congestion, swollenness, pigmentation, moistness, and whether or not the vulval aperture was open.

ii) The presence or absence of a discharge from the vulva and the macroscopic appearance of this excretion when present. Occasionally, some of the excretion was taken for microscopical examination.

iii) The state of the vestibular and vaginal muscles, i.e., whether they were relaxed or contracted, as judged by the ease or otherwise with which the instruments were introduced.

iv) The condition of the external os of the cervix uteri. A rough idea of the condition of relaxation or contraction of the external os could be obtained by exploration with the glass rod.

3. HISTOLOGICAL TECHNIQUES.

As routine fixatives for material destined for histological examination, Heidenhain's "Susa" combination and Allen's P.F.A.3. modification of Bouin's/

Bouin's fluid, were used. Regaud's formol-bichromate either alone or followed by osmic acid was used when cytoplasmic structures were to be studied and either saturated corrosive sublimate in normal saline or absolute alcohol for the study of the distribution of mucin. Zenker and Zenker-formol were used occasionally. Where only anatomical features were to be preserved 5% Formalin in saline was used. Paraffin embedding was used throughout. Sections were cut at 4-6 μ: for cytological detail at 2 μ, and for mucus distribution at 8-10 μ.

The routine stains were Delafield's haema-:toxylin and eosin, Ehrlich's H. & E., and Heidenhain's iron haematoxylin (24 hour method), either alone or followed by van Gieson's connective tissue stain. The Giemsa stain was used for study-:ing the distribution of blood cells. For mucus distribution either Hoyer's thionin method or Mayer's mucicarmine (after Delafield's or Heidenhain's haematoxylin) was employed.

Various other special techniques were employed from time to time: these will be referred to in connection with the descriptions of the organs for which they were used.

The details of the histological methods used are all as given in McClung's "Handbook of Microscopical Technique" (110).

II : THE DURATION OF FERTILE LIFE.

1. THE AGE AT PUBERTY.

The lambs of all the breeds of sheep kept at the Institute were found to become sexually mature in the autumn of the year of birth, i.e., at about 6 to 8 months of age. This appears to be the rule for all British breeds of sheep and for all those kept in North America. Many sheep breeders in the latter country are in the habit of mating their ewes at this age. A few farmers in Britain also do this but the practice is generally deprecated because it interferes with the growth of the animal, which is very far from completed at this age. Dorset Horn lambs born in the autumn come into season in the following spring. Lambs of other British breeds born exceptionally late in the year do not come into season until the autumn of the following year, i.e., until about 15 months of age. The early-maturing races of sheep such as the Hampshire Down, probably reach sexual maturity at an earlier age than slow growing breeds like the Highland Blackfaced, but comparative data are lacking.

Schmaltz (114) gives about 6 months as the age at which Central European sheep reach puberty.

Alongi (4) and Sanctis (141) on the other hand, state that the sheep of Sicily become sexually mature at 12 months of age and those of the Umbria district of Italy at 18-24 months. The difference between the two areas is ascribed to the higher temperature of Sicily. This remarkable difference between the Italian sheep and the great majority of domesticated breeds may be due to the relatively unimproved state of the sheep in Italy. Wild sheep certainly do not breed during their first year; but the Shetland sheep at the Institute were found to be not apprec-:iably later than other breeds in reaching puberty and the Shetland is certainly a closer relative of the wild sheep than most of the domesticated breeds. The difference cannot be due to the time of year at which the lambs are born for in Umbria, at least, this time is the same as in Britain. Alongi states that the cow becomes sexually mature when kept in the stall at 12-14 months: this is also a somewhat greater age than in this country. The native cattle of Kenya do not reach puberty until at least three years of age (Anderson (6)).

2. SENILITY.

It has been shown by Pearl (129), Bell (14), Jones and Rouse (77), and others that the fecundity of a ewe falls off slowly after about five years of age./

age. It may be doubted, however, if a ewe ever becomes completely barren with increasing age so long as ordinary bodily health is retained, and the decrease in fecundity may be due to deterioration in "condition", perhaps because of loss of teeth. Unfortunately, data on the point are difficult to obtain for practically all ewes are killed off relatively very early in life: ewes are almost invariably culled from a breeding flock before eight years of age and ewes which are not bred from are sent to slaughter at a much less age. Pearl (129) records the breeding history of a ewe which continued to breed for 17 years. Smith (153) records an apparently authentic instance of a ewe kept as a pet which, at the age of twenty-one, was then raising her thirty-fifth lamb.

Although genitalia from old "cast" ewes, culled from breeding flocks, formed a large proportion of the material examined at the abattoir, no single instance of a ewe which did not show corpora lutea in the ovaries was encountered during the peak of the breeding season. One of the ewes in the experimental group (705) was known to be at least eleven years old and may have been much older.

III : THE GROWTH OF THE REPRODUCTIVE ORGANS FROM BIRTH TO MATURITY.

The infantile reproductive system of the ewe is characterised by the relatively enormous size of the vagina in comparison with that of the uterus. From birth to puberty the vagina grows steadily, and this slow increment is continued after puberty until bodily maturity is reached. The vagina at birth is about half the length of the full-grown vagina. The uterus, Fallopian tubes and ovaries on the other hand, pass through a phase of very rapid growth shortly before puberty. The uterus of the new born lamb is about $1\frac{1}{2}$ inches in length and each horn about $\frac{1}{4}$ inch in diameter. The ovaries are of comparable size, being little more than $\frac{1}{4}$ inch long. There are no follicles visible to the naked eye, either in the intact ovary or in gross sections. For about the first three months growth takes place at a somewhat slower rate than in the vagina. Thereafter growth in greatly accelerated and at the time of puberty uterus and ovaries have reached a size not far short of that found in the mature ewe, i.e., each horn of the uterus is about 6 inches long and $\frac{3}{4}$ of an inch in diameter, and the ovaries are about $\frac{3}{4}$ of an inch long. Large follicles, comparable in size to those found during interoestrum in the mature animal, are/

are present in the ovary a month or more before puberty. Hammond (66) remarks upon a similar condition in the calf.

In the uterus, the cotyledons (i.e., the special areas of the mucosa to which the foetal membranes become attached during pregnancy) are developed during intra-uterine life. The uterine glands, however, do not develop until the pre-pubertal growth period. A section through the uterus of a new-born lamb is shown in Fig. 17 (page 164). Apart from the absence of glands, the chief differences in structure as compared with the mature uterus are in the loose character of the sub-epithelial mucosa, and in the dispersion of the pig-:ment cells throughout the mucosa.

In comparison with the pre-pubertal growth phases of the uterus in such forms as the rat and mouse, the period in the sheep is protracted, but the growth achieved during the period is comparable in amount.

II : THE BREEDING SEASON.

I : INTRODUCTION.

It is one of the fundamental characteris-:tics of life that reproductive activity is discon-:tinuous, reproductive phases alternating with phases of complete or partial reproductive quiescence; the relative durations of the two in any one species being, to use general terms, determined by environ-:mental conditions. The actual controlling factor in the environment varies widely in different forms, from such apparently simple things as food supply to relatively obscure causes like the lunar phases, which appear to control reproductive activity in the Palolo worm of the Atlantic (108) and some other marine organisms (5, 46). So far as may be judged from what little is known of the reproductive pro-:cesses of wild animals, the mammals adhere without exception to this principle of discontinuous repro-:duction, when they are exposed to the normal season-:al changes in the environment. Zuckerman (170) believes that many of the higher Primates show continuous reproductive activity but many other authorities would dispute this conclusion. When kept under fairly constant environmental conditions, as is the case with the "laboratory" rat and mouse kept in heated quarters, anoestrum may be largely eliminated, although some trace of a breeding season may/

may usually be distinguished. The same end has been attained in some cases by domestication. Domestic cattle may be bred at any season; but here again a trace of a breeding season remains, for it has been shown by Hammond (66) that cows breed more readily during the summer months than during the winter, while Alongi (4) has shown that the cattle of Sicily breed only from January to July. Anderson (6) has found that the maximum number of matings in the native cattle of Kenya, living under wild conditions, occur during the dry season. Even in the human female, in which oestrous periodicity has been very largely obliterated, a marked rise in fre-:quency of conception in the spring months is well recognised (Mayo-Smith (109) and many others) while in the Eskimo woman menstruation ceases altogether during the arctic winter (92).

The seasons of the year at which the various mammals come into "heat" vary greatly from one group to another, but in each single case the breeding season and the duration of gestation seem to be so adjusted as to ensure that young shall be born at the time of the year most favourable to the young animal. In the rat, mouse, rabbit, etc., the breeding season occupies the spring and summer months: gestation being short, the young are produced during the same season. Sheep, on the other hand, breed during the autumn/

autumn and the five months gestation period leads to birth of the young in spring. In cattle, a summer breeding season and nine months gestation lead similarly to birth in spring. The adjustment is more perfect in herbivorous animals than in the carnivores, probably because the former are much more dependent upon the seasons for their food; but the relation is still patent in carnivores, for example, the wild dog breeds only in winter, that is, pups are born in early spring. In a few exceptional cases the same end is attained in a different manner, i.e., by delayed development. In some bats the sexual season is in autumn, but ovulation and development of the foetus are delayed until the following spring: in European roe-deer fertilisation takes place during the autumn, but the development of the foetus is very slow during the first 4 to 5 months (Sakurai (140)).

It thus appears probable that, in the evolution of the mammalian species, breeding season and duration of gestation have together been subjected to natural selection, animals in which the two had been favourably adjusted having a greater chance of perpetuation than those in which an adjustment prejudicial to the survival of the offspring had been established. But it is more difficult to conclude as to which character, breeding season or duration of/

of gestation, has been most subject to alteration through natural selection. Heape (71) favoured the view that variations in duration of gestation and lactation were alone responsible for ensuring the weaning of the offspring at a favourable season; the recurrence of the breeding season being controlled mainly by climatic influences.

Heape's conclusions were based largely upon such admittedly exceptional forms as the roe-deer, in which a different mechanism - delayed development - comes into play to ensure the birth of the young dur-:ing the summer without altering the breeding season. Marshall (106) and others have been unable to concur entirely with Heape's opinions on this subject and to the present author also, they seem untenable. Heape believed that, from the evolutionary standpoint, the breeding seasons of mammals were a primary develop-:ment and the differences in duration of gestation and of lactation secondary adjustments brought about by the necessity for launching the young on their own resources at a favourable season, and at a later stage in development in the case of herbivorous animals of roaming habit.

A differential response to climatic conditions in the onset of the breeding season could have been developed, and maintained when once develop-:ed, only if there was some advantage to be gained by the/

the particular adjustment of each species, and consequently continuous selection against variants. But since the sheep, for example, is indigenous to the uplands of the northern temperate regions, where food is scarce and conditions generally very severe during the winter months, the carrying of a lamb dur-:ing these months must constitute a decided strain upon the pregnant animal and lead to frequent deaths. There must therefore be a constant natural selection against autumn breeding and unless this characteris-:tic is so firmly rooted in the stock as to defy eradication, or unless some more potent factor directs selection towards autumn breeding, a gradual shift in the breeding season must inevitably occur. The re-:quisite "more potent factor" is to be found, in the author's opinion, in the needs of the offspring: it is disadvantageous for a ewe to be pregnant during the winter undoubtedly, but it is more disadvantageous for a lamb to be born in the autumn, or winter. Ad-:mittedly, wide differences in the duration of gesta-:tion have been evolved, but they have been evolved rather because of the necessities imposed by the habits of the species than because the mothers happen-:ed to breed at a time of year which made it essential for them to carry their young over an increased period. The rabbit protects itself against its enemies by burrowing; its young are born in the burrow and are completely/

completely dependent upon the mother for several weeks after birth. There is here, little or no necessity for prolonged gestation. The sheep, on the other hand, lives on the open hillside: its defence against predatory enemies lies in its fleet-:ness of foot. The lamb, accordingly, is born at a much later stage in development and is able to run almost as fast as its mother a few hours after birth. With the increase in duration of gestation it became necessary either to increase the rate of development or to set back the breeding season in order to counteract the adverse tendency towards late birth. No doubt both corrective mechanisms have been invoked but setting back the breeding season has been the principal means in the sheep at least.

It is possible, of course, to argue in the reverse sense, i.e., that the spring breeding season of the rabbit has not necessitated a prolonged gesta-:tion, that the shortness of gestation has meant birth at an early stage of development and that this has decided the burrowing, nest building, habit of the animal, while in the sheep, autumn breeding led to long gestation, the possibility of birth at an advanced stage and therefore freedom to develop into an animal dependent upon fleetness instead of burrow-:ing. But in this, as in all evolutionary problems it is necessary to chose the most reasonable of the possible/

possible explanations and this condition, in the opinion of the present author, is satisfied by the former explanation.

Peculiar conditions like sperm storage in the bat and delayed development in the roe-deer may almost undoubtedly be regarded as secondary condi-:tions called into play in response to some change in the environment, possibly initiated by migration. Moreover, adequate confirmation of the existence of these phenomena has never been obtained.

II : THE BREEDING SEASON IN DOMESTICATED SHEEP.

While there is a great deal of recorded information of a general character regarding the breeding season of domesticated sheep, there are very few detailed records to be found in the literature.

All the domesticated breeds, so far as is known, are polyoestrous, that is, their breeding season consists of a series of oestrous periods re-:curring at short intervals - approximately 17 days. In the great majority of breeds there is only one breeding season in each year, occurring in the autumn, but in a few breeds, such as the Dorset Horn, and a number of foreign breeds, there is a further season in the spring, occurring during the course of lactation if the ewe has been bred in the previous autumn. The Merino, under favourable conditions appears to come into heat almost continuously throughout the year. Thus Quinlan and Maré (130) in observations on the South African Merino covering a period of $7\frac{1}{2}$ months and commencing, apparently, in late September, found that these ewes came into heat fairly regularly throughout the course of the investigation, although the figures given show that there was a quite definite tendency to go into anoestrum towards the end of the period (May). Küpfer (86) on the other hand, in observations on the same/

same breed of sheep in the same locality found that the ewes went into anoestrum during spring and summer, the breeding season commencing about the beginning of January (late summer). That Küpfer's observations are not representative of the normal condition in these animals is shown by the fact that South African farmers are accustomed to put the rams with Merino ewes in October, when the spring rains have commenced - a time when according to Küpfer, the ewes are still in anoestrum. Also, as Quinlan and Maré have observed, Merino lambs are most commonly born in March, April and May in South Africa, i.e., at a time corresponding to service in October to December. Quinlan and Maré ascribe the clearly exceptional character of Küpfer's findings to the circumstance that the year in which his observations were made was one of drought, the spring rains being delayed. Ovarian activity, they believe, was checked by the poor condition of the pasture. In Australia, Merino lambs may be born at any time of the year: there are, however, no accurate observations to show whether there is any period of lessened ovarian activity. The Merino ewe in England is able to produce two crops of lambs, so that there is a spring breeding season. Whether ovarian activity continues throughout the year when the ewes are not mated does not appear to have been determined.

Precise/

Precise data are likewise not available for the American Merino but for the "hothouse lamb" trade Merino ewes are frequently put to the ram in June so that the breeding season certainly commences earlier in these ewes than in most. On the other hand, it is possible to breed in June only if special care in feeding, etc., and selection for early breeders, are practised.

The Persian-woolled sheep of South Africa were found by Küpfer to have a breeding season extending from January to June and to show complete ovarian quiescence during the spring and early summer. Küpfer's observations were made at the same time as those upon the South African Merino: these sheep have not been studied during other years and it may be, therefore, that the season studied was shortened by adverse climatic conditions, as in the Merinos. According to the same author, Central European sheep (Swiss, Steirer and Bergamasker breeds) come into heat regularly throughout the year.

Data have been presented by Alongi (4) to show that the sheep of Sicily come into heat throughout the year although with greater regularity in September and October: the sheep of the Umbria district of Italy, on the other hand, come into heat only during September and October. Alongi ascribes this difference to the higher average temperature in Sicily but it is uncertain whether he refers to the same/

same breed of sheep in the two localities.

McKenzie and Phillips (112) found that the breeding season in pure bred Shropshire, Southdown and Hampshire ewes at the Missouri Experiment Station commenced at the end of August, the Hampshires being slightly earlier than the other breeds. The duration of the breeding season was not determined. The ewes studied were receiving extra food in the form of grain.

Cole and Miller (40) found that sheep (the breed is not stated) kept in California first came into heat at the beginning of September and continued to come into oestrus until the end of February.

Of the breeding seasons of British sheep accurate knowledge has hitherto been almost entirely lacking. With the exception of the Dorset Horn all breeds have ordinarily only a single breeding season, commencing in late summer or autumn. Dorset Horn sheep will mate also in the spring and a short spring season occurs sometimes also in a few other breeds notably the Derbyshire Gritstone, the Limestone sheep of Derbyshire and Westmorland (101,106), the Hampshire Down, the Suffolk Down and the Shropshire. Accord-:ing to Marshall (101) the Highland Blackfaced ewe comes into heat at least three times, and under favourable circumstances five or six times, when kept on lowland farms. He also quotes other authorities to/

to show that under Highland conditions there are only two heat periods.

In the south of England it is customary for farmers to put the ram with the ewes at about the end of August and in some parts of the country where Down breeds predominate, as early as late July. Formerly, it was usual to serve Dorset Horn ewes in June, i.e., in the spring breeding season, but the autumn season is now used by nearly all Dorset Horn breeders. Further north and on high ground, it is customary to put the rams out later in the year and on Scottish Highland farms this is sometimes not done until late November. These differences are largely due to dif-:ferences in methods of husbandry; late lambing is necessary on Highland farms for lambs born before March have a poor chance of survival: while on low-:land farms, especially in the south of England it is profitable to produce lambs in January. But differ-:ences certainly exist between the sheep themselves for it is usually impossible to breed a Highland Blackfaced ewe before October. To what extent these differences are due to genetic differences intensified by conscious or unconscious selection for "early breeders" in the south, and to what extent to climatic or nutritional differences, is debatable.

THE/

THE BREEDING SEASON IN SCOTTISH DOMESTICATED SHEEP.

The direct observations of the present author on this subject bear solely upon conditions in sheep on lowland ground. Data were collected from the stock of ewes kept at the Institute and refer to a number of breeds. This information has been supplemented, however, firstly by information provid-:ed by Highland sheep farmers and secondly by the observation of ovarian activity in slaughter house material. It has been pointed out that although the ewes slaughtered at the Edinburgh abattoir come mainly from Lowland farms, there is always a fair number from Highland farms, and frequently from the north of Scotland. The only district not usually represented is the south-west.

A. The Duration of the Breeding Season.

Six of the experimental ewes employed for observations on the character of the vaginal secre-:tions were observed throughout their breeding season. Particulars of the breeding seasons of these ewes are summarised in Table I. The number of animals re-:presented is too small to justify any general con-:clusions, but it will be noted that in no case was the breeding season less than 9 weeks (5 cycles) and in the longest case it was 27 weeks (12 cycles). A considerable/

TABLE I.

Duration of Breeding Season in Experimental Ewes.

Ewe	Date of first oestrum	Date of last oestrum	Duration of breeding season	Number of oestrous periods.
705: Shetland	28th Nov.	20th Feb.	12 weeks	6
714: Shetland	27th Oct.	28th Mar.	22 "	10
816: Cheviot	29th Oct.	4th May	27 "	12
773: Cheviot x Shetland	21st Nov.	1st Mar.	15 "	7
333: Lincoln x Blackfaced	28th Oct.	17th Jan.	12 "	6
751: Gritstone x Blackfaced (18 mths. old)	12th Nov.	17th Jan.	9 "	5

considerable degree of individual variation is indicated but it appears doubtful whether any of the Institute sheep have breeding seasons much under two months in duration and probably most of them greatly exceed this duration. It may be noted also that the minimum duration found is the same as that given by Marshall as the maximum for Blackfaced sheep on low-ground farms "under unusually favourable conditions".

The ewes recorded in Table I received only pasturage until the end of September. From that time on their food was supplemented by turnips, hay, grain and cake, but only "maintenance requirements" were fed, i.e., the ewes could not be regarded as "flushed". The extra feeding was discontinued at the end of March when the grass had commenced to grow.

Two other ewes of the experimental group (704 and 701) were served by a fertile ram at the sixth heat period (on the 1st and the 19th of February respectively) and both subsequently gave birth to twins.

More extensive information on this subject has been acquired from observations on ovarian activity in slaughter house material. Each time that material was collected at the abattoir an unselected sample of ewe genitalia consisting, on the average, of about 40 uteri was examined. The genitalia were collected from all the killers and came therefore from/

from a number of distinct flocks: in this way wide ranges of breed, age and locality of origin were covered. The percentages of the non-pregnant ewes showing in their ovaries either ripe follicles about to rupture or developing corpora lutea, at each sampling over a period of 13 months, are plotted in Fig. 1. Allowance should be made for two slight errors in these data: at the commencement of the breeding season a few ewes approaching ovulation for the first time will inevitably have been classed as having inactive ovaries since there is no very great increase in the size of the follicle above the size in late anoestrum until a few hours before ovulation: the figures at the commencement of the season are therefore slightly low. Conversely, at the end of the season a few ewes which had ovulated for the last time and gone into anoestrum but still showed large corpora lutea in the ovaries, will not have been distinguished from ewes in late dioestrum: the figures at this season are therefore slightly high.

From this graph it may be seen that ovarian activity commenced to appear in this particular breeding season early in September: from this time until mid-November there was a steady increase in the percentage of the ewes showing ovarian activity. From the 18th of November until the 20th of January, inclusive, every ewe examined had active ovaries (a total/

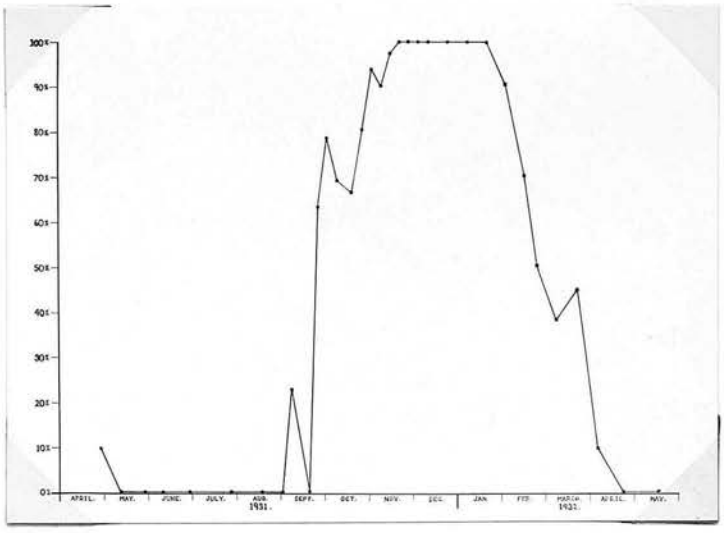


Fig. 1. The Duration of Ovarian Activity in Scottish Sheep. The figures plotted are the percentages of non-pregnant slaughter house ewes showing ovarian activity on successive dates over a period of thirteen months. Each plotting represents about 40 ewes.

total of 397 ewes was examined during this period). From the latter date onwards there was a steady decline of activity until late April when all non-pregnant ewes showed quiescent ovaries. In the previous summer complete absence of ovarian activity lasted from mid-May until the end of August. It may be stated therefore that all Scottish sheep in good condition, irrespective of breed, age and locality of origin, were in a state of ovarian activity from mid-November until January in the year of the investiga-:tion. When it is stated that this condition is "irrespective of age" it must be remembered that very few sheep under 6 months of age are slaughtered at this time of the year. Had mature animals only been considered the period would have extended for almost two more months, from mid-October until mid-February, for after the 14th of October all the "inactive" genitalia came from ewe lambs, i.e., ewes 6 to 9 months old, coming into heat for the first time: this was the case, also, after the peak of the breeding season until the 17th of February. Active lamb ovaries did not appear until the 28th of October and disappeared after the 9th of March.

It is not possible to say whether the season studied was entirely typical since observations were not commenced until January 1931 and ceased in June 1932. However, on January the 7th, 1931, 98% showed/

showed ovarian activity; the percentage declined steadily until May the 13th when 0% was reached. The end of the 1930-31 season was thus identical with that of the 1931-32 season. Meteorologically, the 1931-32 winter was milder than usual.

The commencement of ovulation, it will be shown later (p.85), does not usually coincide with the commencement of the breeding season but precedes the latter by either 17 or 34 days. In a few cases also, ovulation may take place 17 days after the last heat period.

It may be deduced therefore that Scottish ewes when mature (i.e., over 16 months old), come into heat regularly from the end of October until the end of February, a period corresponding to about 7 or 8 oestrous cycles. In lambs (i.e., ewes 6 to 9 months old, born in the preceding spring) the period is much shorter, from mid-November until mid-January, (about 4 oestrous cycles). These figures, however, are minima: some mature ewes come into heat in mid-September: since ewes do not commence to go into anoestrum until late February such ewes must have breeding seasons at least 5 months in duration. Similarly, a few ewes do not go into anoestrum until mid-April: since all ewes are breeding by the end of October these also must have a season at least 5 months in duration. The modal durations are probably about/

about 5 months in mature animals and 3 months in lambs breeding for the first time. These figures correspond to about 8 to 9 and 5 to 6 successive heat periods respectively. The extreme possible duration indicated by the slaughter house material is 7½ months so that ewes such as experimental ewe 816, in which the breeding season lasted for nearly 7 months, are probably rare.

It will be recollected that Marshall (101,106) quoting figures given to him by breeders, states that on Highland farms the Scottish Blackfaced ewe comes into heat only twice while on lowland farms, under unusually favourable conditions, the same breed might show 5 or 6 successive heat periods. That these figures are undoubtedly far below actuality is shown by the data presented above: the figure given by Marshall as the maximum is probably the minimum. Blackfaced sheep, both from Highland and from Border hill farms, constitute a considerable proportion of the ewes killed at the Edinburgh abattoir.

B. Factors affecting the Duration of the Breeding Season.

i) Age.

Lambs coming into heat for the first time at 6 to 8 months of age have a considerably shorter breeding season than older ewes. Comparative figures have/

have already been given. No differences were distinguished between 18 months old sheep in their second breeding season and older ewes.

ii) Breed.

The existence of breed differences in the duration of the breeding season is indubitable. Merino and Tunisian ewes appear to be able to come into heat continuously throughout the year, at least when kept under favourable conditions. The same appears to be true of the Swiss, Steirer and Berga-:masker breeds of Central Europe (86) and of the sheep of Sicily (4). The great majority of the breeds, however, have definitely seasonal breeding periods, as is the case also with wild sheep. The increased breeding capacity of the Merino at least, is definitely attributable to a breed characteristic and not to environmental differences for this capacity is exhibited in America, Britain, and South Africa where other breeds show strictly seasonal oestrum. Whether this great reproductive power was possessed by the original Spanish Merino or was developed during the improvement of the breed, appears to be unknown but the Merino flocks kept in Hampshire in the early nineteenth century were "tupped" in July ((7) p. 130), a time of the year at which only the Dorset Horn among British breeds, will normally accept the ram.

Such/

Such differences as may have existed bet-:ween the breeds of sheep examined at the slaughter house might equally have been due to differences in locality of origin. All the ewes showing ovulation during September were of one or other of the "lowland" breeds but they all came from lowland farms. Ewes ovulating in March and April were mainly Highland Blackfaced ewes but these sheep constituted an unusually high proportion of the ewes being slaughter-:ed at this time. It has not been possible to obtain direct information from breeders on this point for definite "tupping" seasons are recognised in the various districts: the date upon which the rams are put with the ewes varies little in different flocks and this date nearly always falls well within the breeding season.

It has been noted that the three Shetland ewes in the experimental group all had long breeding seasons, one coming into oestrum 10 times, another 6 times, while the third was served at the 6th heat period, and raised twin lambs. This fact is of considerable interest in view of the statements of a number of authorities, to the effect that wild sheep, even when kept in captivity, are usually monoestrous or at most dioestrous: the characters of the Shetland sheep have probably been less subject to alteration through selective breeding than those of any/

any other British domesticated breed of sheep with the exception of the semi-wild breeds inhabiting the Hebridean and Faroe Islands, and the Shetland is certainly quite a near approach to the wild type.

iii) Nutritional conditions.

The practice of "flushing", that is feeding more, or richer, food to ewes immediately prior to the breeding season in order to increase fertility, is commonly stated to bring the ewes into oestrum earlier than would otherwise be the case. If this is true then it might be expected that poor nutritional conditions would curtail the duration of the breeding season. No controlled observations on this point have been conducted, but the breeding season in the experimental ewes was not noticeably less than that indicated by ovarian activity in slaughter house material, although the experimental ewes were kept throughout on "maintenance" rations and few of them were in "marketable" condition.

iv) Environmental conditions.

According to observations quoted by Marshall (101, 106) Blackfaced sheep on highland farms experience only two oestrous periods in the breeding season, while on low ground farms there may be as many as five or six periods. It has been pointed out, however, that both of these figures are decided-:ly below the actual values. In the slaughter house material ovulation commenced earlier in sheep from lowland/

lowland farms and appeared to continue later in those from Highland farms, but this may have been due to the small number examined for very few Highland sheep were available in September and very few Lowland sheep in March. Moreover, such differences as existed may have been due to breed, for the Highland sheep were predominately Blackfaced ewes while very few of this breed came from lowland farms.

C. Factors Affecting the Time of Onset of the Breeding Season.

To the bulk of Scottish sheep breeders the factors controlling the onset of the breeding season are a matter of little practical interest, since the earliest date on which lambs may be born without considerable risk of extensive losses from exposure, corresponds to a service date well within the limits of the breeding season. But in countries with less rigorous climates it is practicable to rear lambs during the winter months and to supply the market with fat lambs at a time when these are scarce and command a much higher price than is ordinarily the case. This practice has gained considerable prominence in America during the past ten years, where it depends mainly upon the high degree of re-:productive activity in Merino and Tunisian ewes and their crosses with other breeds. In the South of England as in America, the spring breeding season of the/

the Dorset Horn is sometimes utilised to produce lambs in autumn to be fattened for the Christmas market. The Down breeds, particularly the Hamp-:shire and the Oxford, are served in August in the South of England so that lambs may commence to be born in early January, and sent to market from April onwards. Tupping of the English longwoolled breeds commences in late September and of the Mountain breeds in late October or November.

It is possible that, if the onset of the breeding season could be controlled, the rearing of lambs through the winter months to supply the market during the first four months of the year, might be practised with profit in England and perhaps also on lowground farms in South-west Scotland, although the principal objections of farmers to early lambing are based upon the high cost of rearing lambs when natural food is scarce.

An attempt has been made, so far as the available data allow, to evaluate the influence of the genetical and environmental factors which might be expected to affect the time of onset of the breeding season.

i) Breed.

The existence of considerable breed differ-:ences in the time of onset of the breeding season cannot be doubted. The Merino, it has been noted, can/

can be bred in June in America, whereas most of the breeds do not come into season until the end of August or September. In Australia (43) and in America crossbreds between Merino ewes and Lincoln, Leicester, Border Leicester, or Romney Marsh rams are used as dams in the production of early lambs, mating taking place in November (early summer) in Australia and in June in America. Thus it would appear that the capacity for early or continuous breeding is transmitted to these ewes by the Merino mothers and in this loose sense, "early breeding" appears to behave as a genetic dominant. It may be noted, however, that Thilo (159) found that while Merinos would mate in June and Leicesters not until Septem-:ber to October, crossbreds between these two breeds could be bred in August but not earlier.

The material collected at the abattoir did not indicate any marked differences in the time of onset of the breeding season in the various Scottish breeds. The ewes in which ovulation took place dur-:ing September were all of one or other of the low-:land breeds but hill Cheviots and Blackfaces were very scarce at this time. Crossbreds between "half-:bred" (Cheviot ♂ x Border Leicester ♀) ewes and Oxford Down rams - quite a common combination in Border flocks - did not commence to ovulate before other lowland breeds. It was impossible also to distinguish/

distinguish between breed differences and differences due to climate, altitude and methods of management.

In the autumn of 1932 the entire stock of ewes over a year old kept at the Institute, was utilised in an experiment to determine breed differ-:ences in time of onset of the first heat. The breeds represented were Border Leicester, Cheviot, Highland Blackfaced, Shetland, Welsh Mountain and the Spanish Piebald breeds, and crossbreeds between Derby-:shire Gritstones and Highland Blackfaces, and between Border Leicesters and Cheviots. A few odd sheep of other breeds and crossbreeds were also included in the group. A small proportion of the ewes was subjected to special experimental conditions in connection with a study of possible environmental factors influencing onset of heat, details of which are given below; but since the differences between these ewes and the normal animals were slight and since, also, all the main breeds were included in the experiment, these animals have been included in this survey. The results have been plotted graphically and are given in Fig. 2. In this graph the number of ewes in each of the principal breeds and cross-:breeds which had come on heat for the first time are plotted against Calendar dates. Actual numbers have been plotted and not percentages because numbers are small and differ greatly for the various breeds.

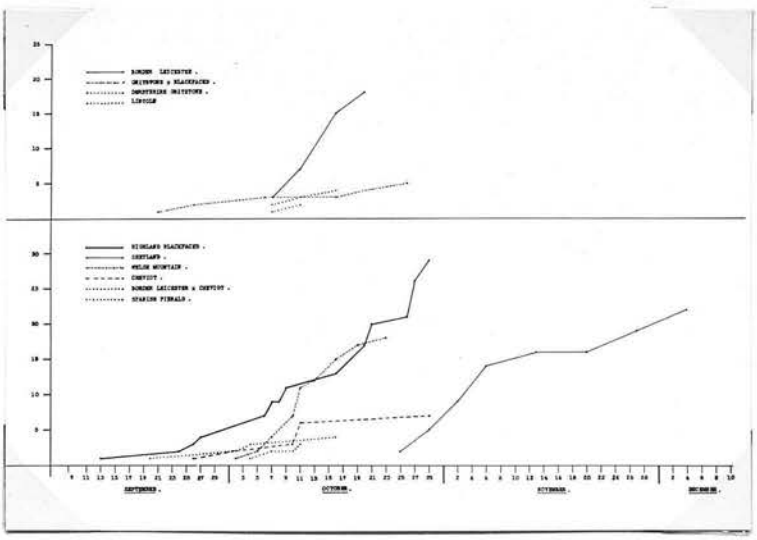


Fig. 2. Breed differences in date of first heat period. The figures plotted are the actual numbers of ewes of each breed which had come into season by the dates indicated. The graphs have been plotted in two figures to avoid undue confusion.

It may be seen at a glance from this figure that there were no significant differences between any of the breeds examined, with one exception, i.e., the Shetlands. All the other breeds and crossbreeds came into heat for the first time about the 12th of October. The Shetlands did not come into season until a month later. In view of this fact it is of interest to note that a single ewe bred from a Shet-:land ram and a Cheviot ewe came into season on the 11th of October; here again, "early breeding" appears to be behaving as a genetic dominant.

ii) Age.

It has been remarked in dealing with the duration of the breeding season that ewe lambs 6 to 9 months old commence to show ovarian activity about a month later than older ewes: presumably the breed-:ing season also commences about a month later. The limiting factor in this case may be age at puberty rather than an actual difference in the date of the first heat period, i.e., January lambs might possibly come into heat as early as mature ewes. There were no discernable differences between 18 month old ewes and older animals.

iii) Nutritional conditions.

The practice of "flushing" ewes before putting them with the ram, primarily to increase fertility, is believed by practical sheep breeders to hasten the onset of heat. This view has been accept-ed by many competent scientific authorities,

(165, 38, 112, 73, 98, 107, 122). There can be little doubt, therefore, that this opinion rests upon a foundation of fact, although, so far as the present author is aware, the matter has not been subjected to adequate controlled experimental verification. Conversely, bad nutritional conditions, drought, etc., are stated to delay the onset of heat in the sheep. Asdell (10) has shown that rainfall has no effect upon the time of onset of the breeding season in goats and argues from this that nutritional influ-:ences have little effect upon the onset of heat in these animals, since the quality of pasturage depends upon rainfall. Darlow and Hawkins (48) have conduct-:ed a controlled experiment on the effect of drastic nutritional manipulation upon the breeding behaviour of the ewe and have concluded that the time of onset of the breeding season may be influenced through nutritive conditions, but the authors recognise that this conclusion must be tentative for the signific-:ance of the results obtained is doubtful. The present writer is unable to understand upon what basis Darlow and Hawkins come to their conclusion for the published data appear to show no significant differences between the experimental and control groups.

The manner in which the supposed stimulat-:ing action of nutritive flushing affects the repro-:ductive system so as to cause earlier mating has not been/

been determined but it has been suggested that the growth of the Graafian follicles is accelerated. The present author would suggest that "flushing" influences the one or two cycles of ovulation which occur at the commencement of the breeding season, normally without the exhibition of heat, and by increasing the amount of follicular hormone secreted, raises the concentration of this substance in the blood well above the threshold value necessary for the elicitation of the "heat" reaction. Direct evidence either confirming or confuting this suggestion is entirely lacking but certain considera-:tions appear to suggest that the interpretation may be the correct one. On the lowland farms of the eastern Border counties it is becoming quite a common practice to commence tupping in early September, lambing commencing in February. Ewes from these farms contribute largely to the ewes killed at the Edinburgh abattoir and the ewes slaughtered may legitimately be regarded as "flushed" since they are for the most part richly fed, without having reached the stable level of fatness at which ovarian activity is said to again decline. On the other hand it has been shown that ovarian activity in the slaughtered ewes does not commence until mid-September: thus it would appear that in these sheep the commencement of ovarian activity coincides with the commencement of receptivity/

receptivity to the ram. It seems improbable, at least, that the two are separated by an interval of a month as they are in unflushed sheep. Then again, it is clear that, if the first heat period is preceded by two cycles of ovulation even in flushed sheep, then flushing, to be effective, must be commenced at least five or six weeks before the first heat is due to occur, since after the first ovulation the occurrence of oestrus is regulated by the ovarian changes: this is not in accordance with agricultural opinion, for flushing is rarely commenced more than a month before the rams go out.

On theoretical grounds, also, it seems probable that the concentration of oestrin in the blood during the spurious ovulation periods must approach the threshold necessary for the incitement of the mating instinct, for the histological changes in the reproductive tract during these periods approach the intensity found at true oestrous periods.

Another farming practice designed to bring ewes into heat, common in America but not in this country, is to withhold food and water from the ewes for 24-36 hours: the ewes are then supposed to come into season soon after the food is restored. McKenzie and Phillips (112) tested out this idea on a small number of ewes and found it to be without foundation.

Whether/

Whether it is possible to influence the onset of the breeding season through the nutritive state of the animal or not, it is quite clear that simple differences of this type are not the basis of the seasonal restriction of the breeding season.

iv) Environmental and climatic conditions.

It is commonly supposed by sheep breeders - and the view is endorsed by many scientific authori-:ties - that the time of year at which the breeding season commences may be greatly affected by local environmental and climatic conditions; as well as by methods of management: thus Alongi (4) states that the sheep of Sicily come into heat all the year round, while those of the Umbria district of Italy breed only during September and October: he ascribes this difference to the higher average temperature in Sicily. It is clear that the existence of such differences has an important bearing upon the problem of the seasonal influences restricting the breeding seasons of sheep and other mammals, and the establish-:ment and precise definition of such controlling factors is therefore a matter of the greatest scienti-:fic interest. Asdell (10) has shown that the time of onset of the breeding season in goats is correlated with the mean summer temperature, a hot summer (particularly a hot August) delaying the onset of heat, while a cool summer has the converse effect.

Rainfall/

Rainfall has no effect on the onset of heat.

It has been mentioned that the breeding season in the Down breeds of sheep in the south of England commence perhaps as much as two months earlier than in Scottish sheep as a whole. This difference may, of course, be due either wholly or in part to the difference in breed, but the fact that no differences were found between the various breeds of sheep at the Institute in this respect (with the exception of the aberrant Shetland breed) does not support this view. Very few Down ewes are bred in Scotland and it has not been possible to obtain comparative data. Nor has it been possible to compare the data obtained from the Institute ewes with figures for the same breeds under different climatic conditions since accurate observations have not been made in other parts of the country, and it is uncertain to what extent breeding dates on sheep farms are affected by methods of husbandry.

Data regarding the onset of the breeding seasons of sheep in climates markedly different from that of Britain are lacking. So far as tropical regions are concerned domesticated sheep are practically confined to the high ground, so that, although they are not exposed to the wide seasonal ranges of temperature that are experienced in the temperate zones they are exposed to wide diurnal ranges. Also, although/

although there are only slight seasonal variations in temperature and daily duration of daylight, rainfall shows marked seasonal variation and this affects the intensity of illumination and also the heat loss of the animals.

While the ability of the Merino and Tunis-:ian ewes to breed continuously is exhibited in temperate climates this ability may owe its origin to the fact that these breeds were evolved in warm climates.

Icelandic sheep (Ovis borealis) commence to come into oestrus in the latter part of November (164).

There are, apparently, no considerable dif-:ferences in the time of commencement of the breeding season in successive years. Slight differences such as those found by Asdell to exist in the goat, may be present but their detection would require the analysis of a considerable bulk of accurate data and such data is not available for the sheep.

v) Seasonal influences.

There can be no doubt that reproductive activity in the sheep is entirely under the control of seasonal changes in the environment, that is, there is no inherent rhythm of ovarian activity; the circumstance that sheep transported from northern to southern temperate zones alter their breeding seasons so/

so that they breed in the autumn of the new environ-:ment eliminates this possibility. But there are a great number of seasonal alterations in the environ-:ment and very little is yet known of the particular change or combination of changes taking place in the autumn which cause ovarian activation.

The great amount of experimental investi-:gation of the inter-relationship between the anterior lobe of the pituitary and the gonads that has been undertaken during the past decade has rendered it possible to say, fairly conclusively, that ovarian activity in mammals is dependent upon the stimulus of a hormone, or hormones, secreted by the anterior pituitary: in the absence of any evidence to the contrary therefore, it may be concluded that the anterior pituitary of the sheep is quiescent during the summer months and resumes activity in autumn. This conclusion does not simplify the problem in the least, however, for it merely shifts the site of operation of seasonal influences. Also, it is not yet possible to say definitely that the breeding season depends upon a seasonal stimulus to the pituitary for alternatively, anoestrum may be the result of an inhibition of pituitary activity during the summer.

Asdell (10) has suggested that "most mammals breed only in spring and autumn because their body/

body temperature may be too high in summer for follicular development". This principle cannot have the general application given to it by Asdell, however, for in the human being for example, it has been shown that reproductive activity is increased in hot climates and depressed in cold climates. Moreover, in a great many mammals the peak of the breeding season occurs during the summer.

It has recently been shown by Baker and Ranson (12) that breeding activity in British field mice (Microtus hirtus) is controlled mainly or solely by the daily duration and intensity of light to which they are exposed: temperature, and nutritive conditions having little or no effect. Bissonnette (23) has shown that the anoestrous ferret may be brought into reproductive activity by exposure to electric light after dark. The last author has shown (20, 21, 22, 25, 26, etc.) that reproductive activity in the male European starling is conditioned by the daily period, intensity and wavelength of light, but that the correct adjustment of these can induce testicular activity only when an adequate diet is fed (24). Rowan (137, 138, 139) and Moreau (117) believed that increased exercise, whether induced by increased duration of light or otherwise, was the cause of sexual activation in birds, but Bissonnette believes the action of light to be a direct one operating/

operating through the pituitary. The sexual seasons of tropical birds, Moreau believed to be limited mainly by food supply and climate.

There are several alterations in the environment taking place during the autumn which might be expected to influence reproductive activity in the ewe, but no one of them in itself appears to fit all the known data.

a) Falling mean diurnal temperature and the occurrence of frosts.

It is natural that the most obvious of the seasonal changes should be thought by sheep breeders and shepherds to be the influence controlling the onset of heat. Scottish shepherds frequently state that ewes commence to come into season after the first frost of the autumn. Horlacher (73) says "ewes begin to come in heat with the first cool nights of summer".

But there seems to be adequate evidence to show that sheep come into season earlier in the South of England than in Scotland and on low ground earlier than on mountains, whereas the diurnal range of temperature is greater and cold nights occur earlier in the year on northern and mountain farms than in the south, and at low altitudes. In Peruvian sheep a distinct breeding season occurs although there is not much seasonal alteration in temperature. The same is true of the wild antelopes of Africa. There are/

are no wild sheep indigenous to purely tropical regions.

Benedict and Ritzman (19) have shown that the metabolic level of the sheep is little affected by the environmental temperature. Rainfall has more effect upon the heat loss of a sheep than a fall in environmental temperature, for the wet fleece is a poor insulator in comparison with the dry fleece. There are, however, no apparent correlations between rainfall and the onset of breeding in temperate climates. If falling temperature had any primary relation to the onset of heat, ewes might perhaps be expected to come into season after shearing.

b) Changes in the character or amount of food eaten.

Gross inadequacies of diet are well known to reduce, if not to entirely suspend, ovarian activity. Loeb (94) and Papanicolaou and Stockard (123) have shown that partial inanition produces ovarian atrophy and complete anoestrum in rodents. In the years immediately following the Great War, sterility was common in the sheep, goats and cattle of Germany and Hungary (Richter (133)) as well as in human beings. Range sheep are well known to be less fertile than sheep kept under close farm management, and this is ascribed to the poor quality of range grazing. In the more extreme cases of malnutrition sterility is associated with complete anoestrum but in milder forms the ewes will mate but fail to conceive.

Excessive/

Excessive fattening, on the other hand, may also lead to anaphrodisia.

Failure to breed may also be caused by the absence of specific substances from an otherwise adequate diet: vitamin E deficiency leads to sterility in rats, but mating is unaffected. Gross deficiency of vitamin A on the other hand leads to ovarian dysfunction. Deficiency of vitamin B also leads to continued anoestrous and ovarian atrophy (Parkes (127), Marrian and Parkes (99)) but the effect in this case is probably due rather to the state of inanition that deficiency of this vitamin produced than to any primary effect upon the repro-:ductive mechanism. On the other hand Reiss and Péreny (131) found that B-avitaminosis caused continued oestrum in both normal and ovariectomised rats.

Mineral deficiencies may also inhibit oestrus. Hart (68) has shown that the partial inhibition of oestrus in the cattle of certain range districts of the United States is associated with low phosphorous and protein contents of the pastures and high calcium:phosphorous ratios. Anoestrum in these cases is associated with emaciation, however, and may be due rather to the low protein content of the pasture than to phosphorous deficiency or lack of a correct calcium:phosphorous balance. The same author describes laboratory experiments on rats in which analogous/

analogous conditions were produced by the same dietary deficiencies. Eckles, Becker and Palmer (51) attribute the low fertility associated with phosphorous deficiency to infrequency of ovulation.

Calcium deficiency may cause sterility but does not appear to inhibit oestrum. In the rat (Kennedy (80)) ovulation continues normally but the normal uterine changes do not occur under calcium deficiency. In cattle, calcium deficiencies are frequently associated with ovarian malfunction (Frei (56)). Küst (89) has shown that rickets in cattle is commonly associated with nymphomania.

Velu (162) has suppressed the oestrous cycle in rats by feeding an excess of flɔurides, he suggests that similar effects may be produced in the domestic mammals, in regions such as Morocco where there is an excess of flɔurine in the drinking water.

Slonaker and Card (150, 151, 152) and Slonaker (149) have shown that there is an optimum level of protein content, below or above which the reproductive rate in rats is decreased, while animal proteins form a better basis for reproduction than purely vegetable proteins. The latter difference is probably due to differences in amino-acid radicles. Courrier and Raynaud (45) caused complete suppression of the sex cycle in rats by feeding a diet poor in lysine.

While/

While there has been very little investi-:gation, observational or experimental, on the effects of unbalanced diets upon the sex cycle of the sheep, it appears to be most unlikely that deficien-:cies of this type affect the reproductive activity of the sheep under normal conditions of management, except perhaps in a few regions such as Morocco, the arid ranges of America, and possibly some of the mountainous districts of Scotland (calcium or phosphorous deficiency). Certainly, no deficiency of this type can be responsible for the complete an-:oestrum during the summer. Conceivably, the an-:oestrum might be due to the richness of pasturage or to the absence from rapidly growing grass of some hitherto undiscovered substance necessary for reproduction (or, alternatively, to the presence of a substance inhibiting reproduction). This possibili-:ty appears to be eliminated by the wide range of pasture conditions accompanying anoestrum in different parts of the world and in successive years in the same locality. In many parts of the world anoestrum corresponds to a period of drought with exceedingly poor pasturage, the breeding season commencing with the rains when pasturage recovers. In Britain it corresponds (usually) to a period with luxurient growth of grass and its termination to the virtual cessation of growth. The same considerations

contradict/

contradict the suggestion that breeding follows as a long delayed effect of the summer flush of pasture.

There appears to be little doubt that an increase in food supply towards the end of anoestrum can accelerate the onset of heat, but it is not possible to bring the ewes into season outside the normal season. There is evidence to suggest, also, that "flushing" does not accelerate the maturation of the Graafian follicles as has been supposed, but simply intensifies the secretion of ovarian hormones at the spurious ovulation periods (see page 52).

The present author is thus of the opinion that, except where gross inadequacies occur, nutri-:tion may be eliminated as a primary factor controll-:ing the breeding season of the sheep.

c) Changes in rainfall and humidity.

The fact that, in regious such as Britain, the seasonal rainfall fluctuates widely from year to year without affecting the breeding season, would appear to indicate that rainfall is not a primary factor in the limitation of the breeding season, although there is a general coincidence between the onset of the wet season and the onset of heat in certain parts of the world where the distinction between the seasons is more marked in this respect. Anderson (6) has found that the greatest amount of sexual activity in the native cattle of Kenya is in the dry season.

d)/

d) Falling diurnal duration and intensity of daylight.

On purely theoretical grounds, it appears probable that the mechanism of restriction of the breeding season may be found in the seasonal alterations in duration and intensity of daylight, operating either directly upon the ewe - possibly by the photo-synthesis of some compound - or through its effect upon the activities of the animal. This conclusion may well be found to rest upon the inadequacy of the available data on the subject, however. Changes in daylight are certainly the most constantly occurring of the environmental changes and apparently the most nearly related to the onset of the breeding season in sheep and in many other animals. Nevertheless there are certain apparent discrepancies which are at present obscure. In tropical regions the seasonal variations in the diurnal duration and intensity of light are very small, and yet sheep in these regions appear to possess a quite definite breeding season. It is possible that the slight alterations in duration, coupled perhaps with alterations in intensity between the dry and the wet seasons, may be sufficient to form a basis for the restriction of the breeding seasons of the sheep acclimatised to these regions: if this is the case, sheep from temperate zones introduced into tropical regions would be expected to go into anoestrum for a time/

time, until they became acclimatised. Information on this subject is, unfortunately, entirely lacking.

A further discrepancy appears to lie in the earlier onset of heat in sheep in southern and low-:land districts of Britain. If these differences do actually exist, then they cannot be accommodated by an hypothesis which assumes light to be the only factor controlling the onset of the breeding season, for differences in the duration of daylight between high and low altitudes in the same latitude and between north and south of Britain in October, are trifling.

Too little is known of the physiological actions of light to allow even tentative suggestions to be made regarding the mode of its supposed action on reproductive activity, but the stimulus is almost certainly carried through the pituitary. The activities of a ewe are not subject to any marked change with the seasons: there is a tendency to rest at night rather than during the day but the difference is not a clear cut one as sheep will usually graze on a moonlit night. In summer, also, any loss of rest during the night is made up for by sleep during the hot part of the day. It is certainly true, however, that the spasms of violent activity which sometimes overtake a flock do not occur so frequently during the summer. It seems improbable therefore/

therefore that light can be operative through increased activity. Photochemical action through the skin is less likely to occur in the sheep than in other mammals, on account of the density of the fleece, which must cut off most of the light from the skin. By deduction, therefore, it seems that the pituitary is most likely to be affected by light through the medium of the eye, by a nervous stimulus or by photosynthesis of some substance. Photo-:synthesis is already known to take place in the eye; the "visual purple" is formed by such a reaction. It is not improbable therefore that other compounds may be formed in the same way.

As a tentative hypothesis based upon the available data, the author would suggest that the breeding season in the sheep is limited primarily by the diurnal duration and intensity of light, operat-:ing either directly (through the eyes or skin) or possibly through its effect upon the activities of the ewe. In addition reproductive activity is increased in warm climates, in the sheep as in other mammals. Gross inadequacies in nutrition may inhibit breeding and conversely rich feeding may intensify reproductive activities but without disturbing apprec-:iably the restrictions of light and temperature.

The collection of more extensive data on the breeding seasons of sheep in different climates is being undertaken and it is hoped that this hypothesis may/

may be confirmed or refuted in this way. In addition it is hoped to design an adequate experiment on the lines suggested by this hypothesis and by the preliminary experiment described below, and to bring the breeding season of the sheep under experimental control.

III : THE EXPERIMENTAL CONTROL OF THE BREEDING SEASON.

A preliminary experiment upon the lines suggested by the theoretical conclusions arrived at in comparing data from different parts of the world, was staged during the 1932-33 season at the Institute of Animal Genetics, Edinburgh. While the results of this experiment were completely inconclusive they have a certain inherent interest, and suggest the linesupon which further experiments may be carried out: it is proposed therefore to give a brief statement of the experiment and of its results.

The number of ewes employed was restricted to 28 by the limitations of accommodation. The constitution of the five experimental groups into which these ewes were divided and the experimental conditions to which they were subjected, are given in Table II. Group F. was composed of ewes which were being bred from in connection with another in-:vestigation and was therefore composed of ewes of one breed only. The remaining sheep were split up among the other four groups so as to attain as much uniformity of breed and age between the various groups as was possible with the ewes available.

The shed used for the experiment was a well ventilated one about 30 yards in length. It was divided/

TABLE II.

First Service Dates of Experimental Ewes, 1932-33 Season.

Group B : Extra Light - Dusk to 1 a.m.

321	:	Blackfaced	4th October.
399	:	Blackfaced	4th "
333a	:	Border Leicester x Cheviot	4th "
442	:	Gritstone x Blackfaced	6th "
773	:	Shetland x Cheviot	15th "

Group C : Normal Light - Confined 1 p.m. to Dusk.

557	:	Blackfaced	13th September.
517	:	Border Leicester x Cheviot	16th October.
473	:	Derbyshire Gritstone	7th "
460	:	Merino x Cheviot	10th September.
705	:	Shetland	

Group D : Extra Dark and extra Warmth - in Dark Shed, Dusk to 1 p.m.

212	:	Blackfaced	24th September.
229	:	Border Leicester x Cheviot	1st October.
559	:	Cheviot	20th September.
001	:	Gritstone x Blackfaced	21st "
333b	:	Lincoln x Blackfaced	3rd October.

Group E : Extra Dark - in Dark Shed, 1 p.m. to Dusk.

220	:	Blackfaced	26th September.
556	:	Blackfaced	27th "
246	:	Border Leicester x Cheviot	21st "
349	:	Gritstone x Blackfaced	26th "
816	:	Cheviot	5th October.
770	:	Shetland	

Group F : Extra Warmth - indoors, 1 a.m. to 8 a.m.

20	:	Welsh Mountain	1st October.
21	:	" "	5th "
17	:	" "	7th "
19	:	" "	7th "
18	:	" "	11th "
96	:	" "	11th "
99	:	" "	11th "

Neither of the two Shetland ewes had come on heat by the 14th October when the experiment was discontinued.

divided into two halves, one half being darkened while the other was exposed to the southern light. It was not possible to completely darken the "dark" side of the shed without interfering with ventilation and raising the temperature of the dark side above that of the light: this was, of course, undesirable so that a compromise was effected, the temperature never rising more than 5° F. above that of the light side and light being so reduced that on a bright, sunlit day, the intensity of illumination was considerably less than that outside on a moonlit night. There were, however, bright patches on the roof opposite the ventilators.

Groups B. and F. were housed in a separate brick building well exposed to daylight. During the day these groups occupied adjacent paddocks entirely similar in pasturage. Groups C., D., and E. occupied three other paddocks, similar to those occupied by B. and F., but the amount of grass varied considerably and the three groups were rotated from one paddock to another to ensure equal feeding. All ewes received a small ration of hay or grass cut from adjacent paddocks when they went into experimental quarters. No other extra food was given.

The experimental procedures with the different groups were as follows:-

Group B. was driven into the house at dusk and given/

given extra light, provided by a 100 watt lamp suspended 3 ft. above the pen, until 1 a.m. when they were turned out to the paddocks.

Group D. was driven into the dark side of the shed at dusk and released again at 1 p.m. (i.e., noon G.M.T.).

Group E. was driven into the dark side of the shed at 1 p.m. and released at dusk.

Group C. was driven into the light side of the shed at 1 p.m. and released at dusk.

The two "dark" groups thus received about 7 hours less exposure to light per day than the others. The object of having two dark groups was to eliminate any differences which might have been due to temperature differences, one group being exposed to, and the other sheltered from, the minimum temperature period in the early morning.

As a normal control the whole group of ewes kept at the Institute was utilised.

Group F.was run with a fertile ram. For the remaining groups a vasectomised ram was used: this ram was most active and there was no possibility that any ewe showing signs of heat would not have been detected. The abdomen of this ram was "keeled" (i.e., rubbed with paint) and he was run with each group for a duration of time each day in proportion to the size of the group, i.e., he spent most of the time with the main control group.

The/

The experiment was commenced on the 10th of August and discontinued on the 14th of October, by which date all the experimental ewes except the two Shetlands had come into season. It was found subsequently that Shetland ewes are constitutionally "late breeders".

The first service dates of each experimental group are given in Table II and are plotted graphic-:ally in Fig. 3, together with the corresponding data for the big control group.

It will be seen from this graph that all five experimental groups came into season earlier than the control group. Group B. was, on the average, about 9 days early, Group F. about 9 days early, Group C. about 16 to 20 days early, Groups D. and E. about 19 days early. Although the number of ewes in each group was very small there seems to be little room for doubt that there were significant differences between the experimental groups, with the possible exceptions of B. and E.; and the main control group. The interpretation of these differences is another matter: the experimental groups were, in common, subjected to three influences to which the main control group was not subjected, i) more, and regular "handling", i.e., driving them into sheds, etc., ii) confinement, iii) a reduction in food - as a result of confinement.

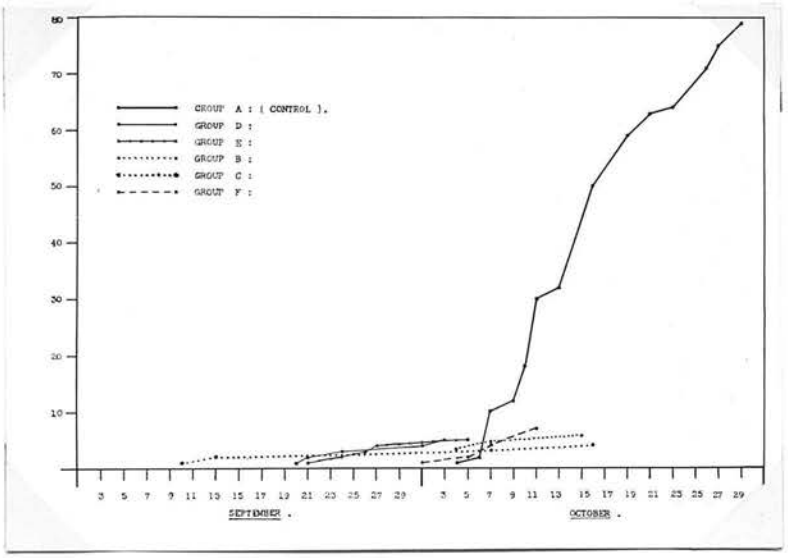


Fig. 3. Graphs showing effect of interference with the environment upon the time of commencement of the breed-:ing season.

It seems unlikely that reduction of food supply can have been the operative factor, although those ewes which were most confined - and consequent-:ly fed least - came into season earliest, for there is fairly conclusive evidence to show that increased, rather than decreased feeding hastens the onset of heat. If simple confinement were responsible for the differences its effect would probably have been wrought through the reduction of exercise that it entailed.

It seems possible that, had the experiment been commenced at a much earlier date, more conclus-:ive results might have been obtained. While the differences between the experimental and control groups amounted to only 2 to 3 weeks at the most it must be remembered that the experiment did not commence until August the 10th and the control ewes commenced to come into season on October the 4th.

It is quite clear that in any further experimentation on the seasonal factors in the limitation of the breeding season the possible effect of confinement, different feeding, etc., cannot be eliminated in the experimental groups, and experi-:mental and control groups will have to be subjected to uniform conditions of confinement and fed a standard diet. The effects of confinement and of artificial diets alone will be determined by separate experiments in which other environmental factors are constant./

constant. It will be necessary, also, to utilise a much more powerful source of artificial illumination and preferably to imitate so far as possible the conditions of "dawn" and "twilight".

Unfortunately, none of the ewes employed in the preliminary experiment were autopsied and vaginal smears were not taken, it is not possible to say, therefore, whether ovarian activity commenced in these ewes before the first heat period.

IV : THE BREEDING SEASON IN WILD SHEEP.

There can be no doubt that the sexual seasons in such animals as wild sheep, goats, and deer are of very short duration and of very definite seasonal occurrence. The commencement of these seasons is a very obvious occurrence because of the phenomenon of rut in the male and the terrific combats which take place between the males for the possession of the females. The time of the sexual season seems to differ considerably in different species of sheep and in different parts of the world, but it appears to occur always in the autumn or winter. The Mufflon sheep, Ovis musimon, of Corsica and Sardinia, perhaps the nearest wild relative of the domesticated sheep, Ovis aries, breeds in December and January according to Lydekker (96) and from March to June according to Sanctis (141). In captivity (at the Zoological Gardens, Rome), it breeds in December and January according to the latter author.

It has become a commonplace statement, that sheep in the wild state are usually monoestrous, or at most dioestrous. Marshall (101, 106) arranges the wild and domesticated species in series and indicates the increasing amount of polyoestrum, attendant upon domestication, from the monoestrous state/

state of many of the wild sheep to the state of continuously recurring oestrum in the Merinos of Australia. So far as the writer is aware, however, there is no evidence to show that any wild species of sheep is monoestrous. Sheep generally are very fertile animals: except in highly specialised breeds, such as the Merino, very few ewes fail to conceive at the first service. Under wild conditions, there-:fore, where males are constantly present in the breeding herds, the ewes must nearly all become pregnant at the first heat period, and heat will, naturally, not recur. The definite establishment of the monoestrous (or polyoestrous) condition in wild sheep could be accomplished, i) by direct observation of a herd with a sterile male - the signs of oestrum in the female are too slight to be detected without the use of a male, ii) by the controlled introduction of males to a herd, or iii) by counting the number of "generations" of corpora lutea in the ovaries of females from a herd not containing males, shot after the breeding season. Even observations with a sterile male might not be reliable since the condition of pseudopregnancy might conceivably be induced and interrupt the cycle of oestrous changes. The author is not aware of any observations that have been carried out on these lines, and the obvious difficul-:ties in carrying out such investigations under truly "wild"/

"wild" conditions, makes it extremely improbable that such observations exist. The red deer of Scotland is also commonly stated to be monoestrous but observations by Miller (114) have shown that two or more "generations" of corpora lutea may be present in the ovaries of hinds during the early stages of pregnancy. Ovulation may therefore occur at least twice in this species although heat does not necessarily accompany each ovulation.

It is probable that domestication and improvement have led, in many cases, to increased polyoestrum, for example in the Merino, but even here the evidence is weak for many semi-wild sheep show a very high degree of polyoestrum. Thus it has been shown that the Shetland breed (admittedly under domesticated conditions) has a breeding season commensurate with that of the Scottish fully domesticated sheep. The sheep of Sicily, which are very "unimproved" and live under semi-wild conditions, breed at any time of the year (Alongi (4)). The semi-wild sheep of Patani breed twice annually (Marshall (101)).

That an increase in reproductive ability can be brought about by domestication and improvement is shown by the high degree of polyoestrum existing in domesticated cattle when compared with their wild relatives. The relatively primative cattle of Sicily still show a restricted breeding season (Alongi (4)).

V : GROSS CHANGES IN THE OVARIES DURING ANOESTRUM AND THE BREEDING SEASON.

Before puberty and during anoestrum the ovaries are, as in other mammals, quiescent. The breeding season is made up of a series of ovarian cycles, each comprising the growth and rupture of a number of Graafian follicles, followed by the growth and subsequent atrophy of "spurious" corpora lutea (or "corpora lutea of oestrous", as distinct from the "true" corpora lutea or "corpora lutea of pregnancy"). Each complete cycle occupies about 17 days and each commences with a "heat" period. Should the ewe be served by a fertile ram and conceive, the spurious corpora lutea are converted into true corpora lutea which persist throughout pregnancy, the growth and rupture of further Graafian follicles being inter-:rupted. Towards the end of pregnancy - which lasts about 21 weeks - atrophy of the true corpora lutea sets in. In all Scottish, and probably in most English breeds of sheep, with the exception of the Dorset Horn, ovulation does not recommence until the onset of the next breeding season, usually about 6 to 7 months later; but in the Merino (130) and presumably in all other breeds of sheep able to raise two crops of lambs in a year, ovarian cycles recommence about 10 to 15 days after parturition, although, according to Quinlan and Maré (130), the ewe/

ewe will not then accept the ram.

In mid-anoestrum, i.e., during the height of the summer, the ovaries are small and compact: they contain a great number of Graafian follicles but these are seldom more than 3 mm. in diameter (see Fig. 4). In mature ewes the ovary contains also a number of small yellow, or brown patches, 2 to 3 mm. in diameter and irregular in shape: these are the remains of corpora lutea developed during the previous breeding season. As the breeding season is approached there is a gradual increase in the average size of the larger follicles. When ovulation com-:mences to take place there are usually about 20 follicles over 4 mm. in diameter, but seldom more than 4 or 5 of these exceed 6 mm. As the breeding season progresses the number of large follicles decreases. There is always a gradation in size of the follicles so that follicular growth appears to be continuous, the difference between anoestrum and the breeding season lying in the stage to which growth proceeds. The condition of the ovaries during the first month or two of anoestrum differs according to whether the ewe bore a lamb during the previous winter or not. During the greater part of pregnancy follicular growth is almost completely inhibited. Shortly before parturition follicular growth re-:commences and two months or so after parturition the number/

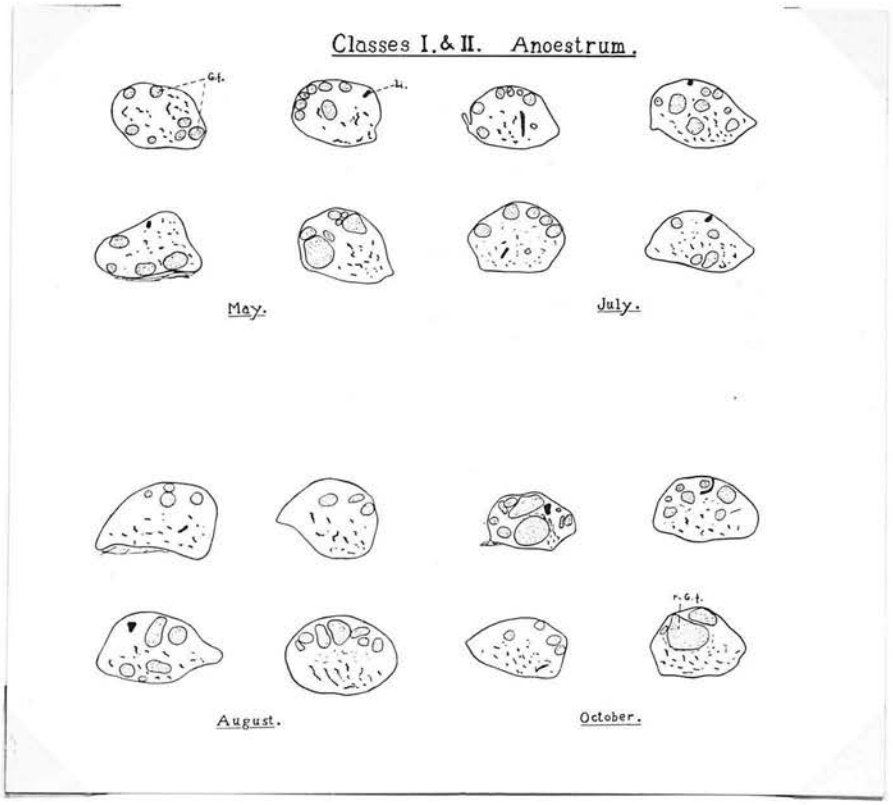


Fig. 4. Sections through ovaries of ewes killed at successive stages in anoestrum. (Diagrammatic). About half natural size.

G.f. Graafian follicles.
r.G.f. ripe Graafian follicle.
li. lipochrome patch, i.e., remains of an old corpus luteum.

number and mean size of the follicles increases up to the usual mid-summer level. In a ewe which has not been pregnant during the past winter, there is a gradual decrease in the mean size of the follicles down to the mid-summer level.

It thus appears that the ability to produce large Graafian follicles is greatest during the early part of the breeding season, decreases towards the end of the season and reaches a minimum about mid-summer: during pregnancy follicular development is almost completely inhibited. It is not yet known whether the follicles produced during anoestrum undergo atresia or are simply arrested in their development. The large follicles present in the ovary during early pregnancy must, however, undergo atresia since they disappear from the ovaries without rupture. There is, however, no macroscopic evidence of atresia. The matter is being studied histologically.

VI : THE OCCURRENCE OF OVULATION WITHOUT OESTRUM.

It has been stated repeatedly in the fore-:going pages that ovulation occurs at the commence-:ment of the breeding season in the sheep, without the exhibition of the mating instinct. The evidence upon which this conclusion is based may conveniently be summarised in this place.

i) Five out of six ewes killed at the Institute between September the 15th and October the 10th, 1932, i.e., during the month previous to the time at which the breeding season might have been expected to commence in these ewes showed, in their ovaries, either developing corpora lutea, or regressing corpora lutea and, in addition, ripe Graafian follicles. The remaining ewe showed a ripe Graafian follicle but no corpora lutea. One out of the five ewes showed ripe follicles and one "generation" of regressing corpora lutea, i.e., had ovulated once and was about to ovulate again; one showed developing corpora lutea only, i.e., had ovulated once; and three showed one generation of regressing and one generation of developing corpora, i.e., had ovulated twice. A highly active vasectomised ram, whose abdomen had been smeared with paint, ran with these ewes almost constantly and would certainly have marked any that came into heat even for a short time, as/

as he searched almost constantly for ewes on heat. Two lambs 6 months old killed in September showed no corpora lutea but lambs do not commence to come into season until the end of October.

ii) The vaginal smear from the ewe shows definite cyclical changes during the dioestrous cycle, the chief change being a marked rise in the number of semi-cornified cells shortly after ovulation. The experimental ewes employed in tracing vaginal changes nearly all showed marked peaks in the semi-cornified epithelial cell content of the vaginal sample, either about 14 days or 14 and 30 days previous to the first heat period (see page 252), i.e., at dates separated by one and two dioestrous cycle lengths from the time of the first peak in cell content following an actual heat period.

iii) Five out of the seven of these experimental ewes autopsied during the breeding season showed in their ovaries a greater number of "generations" of corpora lutea than could be accounted for by the number of heat periods through which they had passed. Two of them showed one supernumerary generation and three of them two supernumerary generations. In the other two ewes the number of generations could not be determined with certainty.

iv) A corpus luteum approximately $3\frac{1}{2}$ days old was found in one of the ovaries of one of the Shetland ewes/

ewes in the 1931 experimental group, the ovaries of which were examined by operation. This ewe had shown a marked rise in the semi-cornified epithelial cell content of the vagina a day before the operation. Heat did not occur until about five weeks after the operation but was probably delayed by the operation since one ovary was removed. Heat certainly had not occurred before the operation.

v) It will be shown that the frequency graph drawn from all the available data on the duration of the dioestrous cycle is distinctly bimodal in nature (see page 99) there being a main peak at about 17 days and a second much less distinct peak at about 34 days. There are also slight indications of 3rd and 4th modes at 50 and 67 days. These modes thus occur at 2,3 and 4 times the interval of the main mode. There can be only one reasonable explanation of these facts, i.e., one or (rarely) more "oestrous" periods may be passed through without the exhibition of heat. Some of the cycles contributing to the higher modes may reasonably be put down to failure to observe a very short oestrous period, but when a "keeled" ram was with the flock constantly this interpretation is not allowable. (The possible errors of the graph are discussed further on page 100.)

Whether or not ovulation occurred at the "missed" oestrous periods, is, of course, a matter for speculation/

speculation but there is good reason to suppose that the corpus luteum regulates the ovarian rhythm - probably through the medium of the pituitary - and it is more reasonable to suppose that a new genera-:tion of corpora lutea was formed than that the life of the old generation was doubled. Unfortunately none of these doubled periods occurred among the author's animals: it is thus not possible to say whether there were any vaginal changes accompanying the supposed ovulation.

The foregoing facts may be taken as evidence that ovulation sometimes occurs during the breeding season without heat and therefore indirectly supports the view that it may occur similarly before the breeding season begins.

Evidence has already been presented to show that under highly favourable nutritive conditions these spurious ovulations may be converted into normal oestrous periods at which the mating desire is expressed.

Observations carried out by Miller (114) have shown that in the red deer of Scotland, hinds in the early stages of pregnancy commonly show in their ovaries not only the corpora lutea of pregnancy but one or more generations of regressing corpora. Since, in the wild state, it is probable that very few hinds fail to become pregnant at the first service,/

service, this fact is suggestive that the phenomenon of spurious ovulation may not be confined to the sheep but may occur in other Ungulates also.

III : THE DIOESTROUS CYCLE.

I : INTRODUCTION.

Terminology.

In dealing with the phases of the dioestrous cycle Heape's terms have been used throughout. It should be understood at the outset, however, that the terms are used purely for convenience in order to refer readily to different periods of the cycle without the repeated use of phrases. No reference to anatomical changes is implied. The oestrous cycle of the ewe can be divided on anatomical grounds into only two phases, oestrum and interoestrum: any attempt to further subdivide the interoestrous period would be unjustified, for the anatomical changes in the accessory reproductive organs are continuous during interoestrum.

"Oestrum" (or "oestrus") has been used in its original sense, i.e., it has been applied to that phase of the cycle during which the female is willing to accept coition with the male. During the last five years or so, this term has been used by many investigators dealing with rodents to refer to the period of the cycle at which cornified cells appear in the vaginal smear. In the opinion of the writer this use of the term is totally unjustifiable. While it is true that cornified cells are present in the vagina of the mouse during only the mating phase of/

of the normal oestrous cycle, Marrian and Parkes (100) have shown that the amount of heat-producing hormone necessary to produce keratinisation of the vagina of the mouse is about a two-hundredth part of that necessary to induce true oestrus. Keratinisa-:tion may also be induced by totally distinct means having no relation to reproductive processes. More-:over, if the term were applied to other animals in this sense it would refer to an entirely different phase of the cycle, for keratinisation in the sheep, for example, is a post-oestrous phenomenon. Had less reliance been placed upon the vaginal smear as an infallible means of detecting oestrum, much of the confusion which exists in sex-physiological litera-:ture might have been avoided.

"Metoestrum" has been applied to the phase of the cycle extending from the cessation of heat until full development in the size of the corpus luteum is reached, i.e., from about the second to the eight day of the cycle, measured from the time of commencement of heat. "Dioestrum" lasts until the corpus luteum begins to atrophy at about the 16th day. "Pro-oestrum" commences when atrophy of the corpus luteum sets in and lasts until heat commences: it thus occupies only about 24 hours at most. Marshall (101) and, more recently, Cole and Miller (42) distinguish between pro-oestrum and oestrum on anatomical grounds: the present author is unable to/

to agree with this division for the "pro-oestrus" phenomena frequently do not commence until oestrum is well advanced.

Another, and perhaps more adequate, division of the oestrous cycle could be based upon the ovarian changes. There are two phases of ovarian function, a "follicular" phase during which Graafian follicles undergo rapid maturation and corpora lutea are either entirely absent or atretic, and a "luteal" phase during which a growing and presumably active corpus luteum is present and follicular maturation is in abeyance. The follicular phase occupies the greater part of the heat period and commences a few hours before heat. The luteal phase occupies the last few hours of heat and the greater part of the interoestrum.

In referring to the hormones of the ovary and pituitary which are concerned in the reproductive processes, the use of particular names has been, for the most part, avoided, except in the case of the heat-producing hormone of the ovary, for which the term "oestrin" has been employed. The physiological properties of this hormone are now fairly well defin-:ed and the term oestrin has received wide-spread adoption. The same cannot be said of the hormones of the corpus luteum and those of the anterior lobe of the pituitary.

II : THE PERIODICITY AND DURATION OF OESTRUM.

1. THE DURATION OF THE DIOESTROUS CYCLE.

The periodicity of "heat" in the sheep has been the subject of investigation by several workers and data are available for a number of different breeds living in several parts of the world. The most extensive figures are those given by Quinlan and Maré (130) for the South African Merino ewe: in their study the lengths of nearly 500 dioestrous cycles were recorded. Unfortunately, the data were not collected with the same degree of accuracy that characterises the records of other observers, the ewes having been examined for oestrous only once a day. Each record is thus subject to an error of ± 24 hours. The range of variation was from 6 to 68 days but the distribution graph shows a strongly marked modal group with a mean duration of 17 days. Eighty-two per cent of the 481 cycles fall into the 16, 17 and 18 day classes. There is a second and much less strongly marked mode at 33-35 days. The average duration of the dioestrous cycle extracted from Quinlan and Maré's data is 18.8 days.

McKenzie and Phillips (112) record the lengths of 116 oestrous cycles in a group of pure bred Hampshire, Shropshire and Southdown ewes kept at Missouri, U.S.A. The figures given should be quite accurate/

accurate since "keeled" rams ran with the ewes at all times and observations were frequent. The distribu-:tion diagram given by these authors shows a range of variation from 8 to 49 days. However, 79% of the periods fall into the 14, 15 and 16 day classes. There is a distinct mode at 15 to 16 days and the average duration is 16.6 days.

Casida and McKenzie (37) observed the durations of 34 cycles in 9 Hampshire and crossbred ewes, also kept at Missouri, U.S.A.; in this case observations were taken four times daily or more frequently. The range of variation found was from 7 to 35 days but 74% of the cycles fell between 15 and 18 days. The modal group at 16¼ days contains 7 cycles (See Fig. 5). The average duration extract-:ed from these records is 15 days.

Darlow and Hawkins (47) report an average duration of 17.3 days and a range of variation from 13 to 21 days in 168 cycles of yearling Merino ewes kept at Oklahoma, U.S.A. Details of the distribu-:tion are not given but nearly one half of the cycles fell into the 17 day class. These observations are subject to the same error as those of Quinlan and Maré in that the ewes were tested for oestrus only once daily.

The data given by the above and by other authors are summarised in Appendix Table II, together with the author's data.

The/

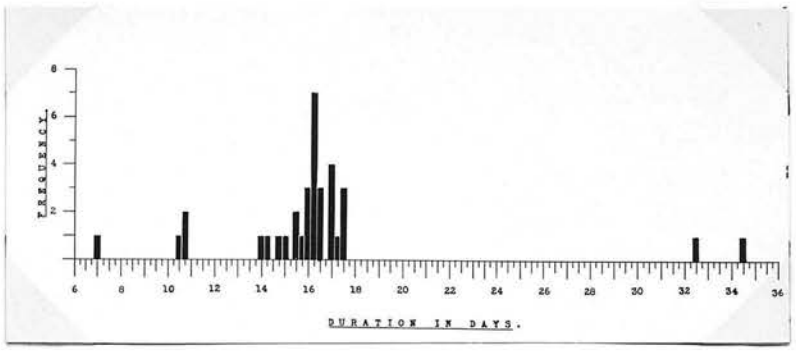


Fig. 5. Duration of the Dioestrous Cycle. Casida and McKenzie's data, represented graph-:ically. (34 cycles)

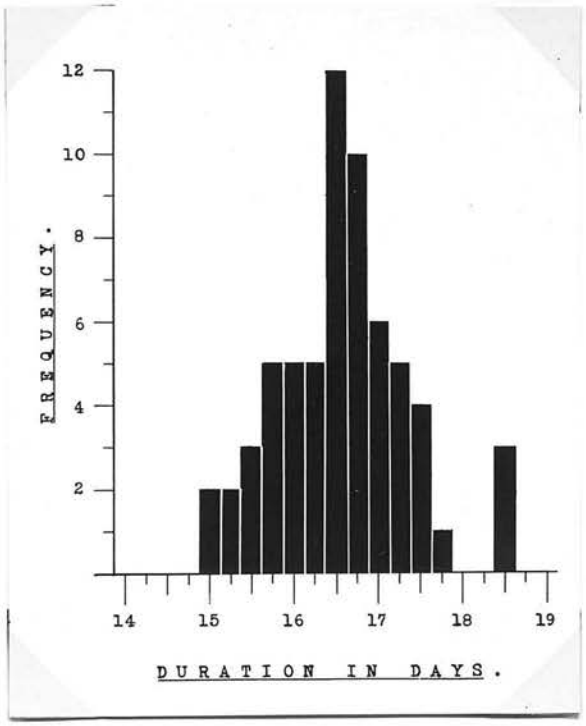


Fig. 6. Duration of the Dioestrous Cycle. The author's data (63 cycles). The mean is 16.4 days and the mean deviation 19.3 hours.

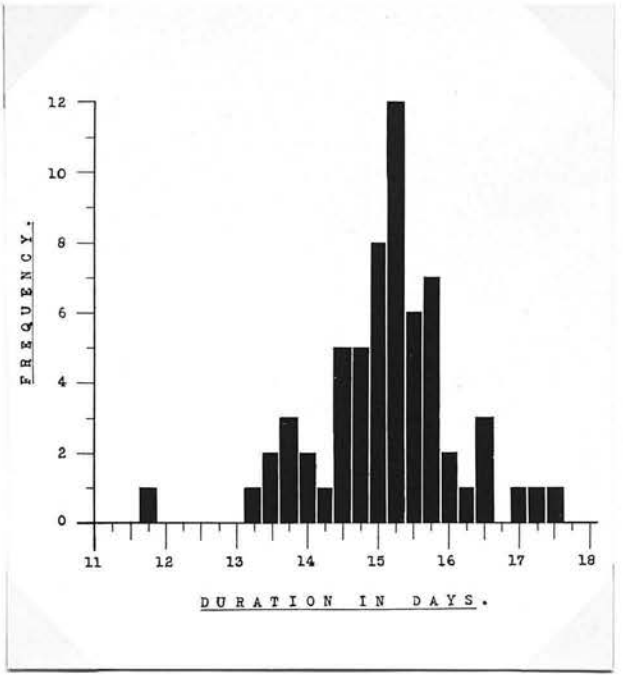


Fig. 7. Duration of Interoestrum. Compiled from the author's data. Mean Duration 15.02 days, Mean Deviation ± 21.93 hours.

The durations of the successive dioestrous cycles in the 17 experimental ewes utilised by the present author are recorded in Appendix Table III: the cycle length has been measured from the commence-:ment of one oestrous period to the commencement of the next.

The distribution of the 63 normal cycles with regard to duration is shown in Fig. 6. All cycles observed after operative interference have been omitted. The fifth and sixth cycles of ewe 816 have likewise not been considered since there is strong evidence that these cycles cannot be consider-:ed as normal (see page 223).

The range of variation shown is considerab-:ly less than that shown by the data presented by other observers. The average duration extracted from these data is 16.4 days, and the mean deviation $\pm$ 0.8 days.

It will be seen from Appendix Table II that there is close agreement between different observers as to the duration of the oestrous cycle, with the exception perhaps of Küpfer (86), Sanctis (141) and Schmaltz (144) whose data indicate a some-:what longer cycle in Central European sheep than has been found elsewhere. There may also be significant differences in the modal duration observed by other observers. McKenzie and Phillips obtained a mode of

15/

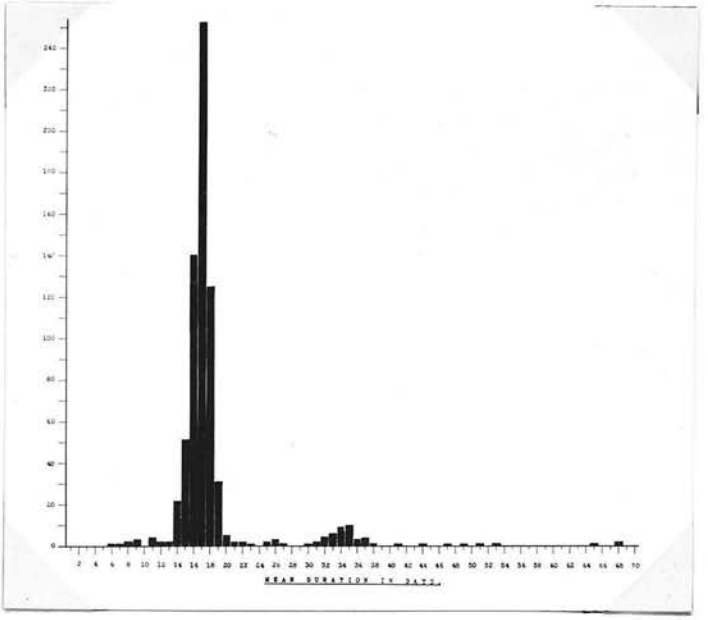


Fig. 8. Duration of the Dioestrous Cycle. The figure has been constructed from the combined data collected by Quinlan and Maré, McKenzie and Phillips, Casida and McKenzie, and by the present author, and represents a total of 697 cycles.

15 to 16 days, Casida and McKenzie 16¼, the present author 16½ to 16¾, Quinlan and Maré 17, and Darlow and Hawkins 17 days. In the last two cases the animals were entirely Merinos. In view of the close agreement between the different sets of observations it has been considered a legitimate treatment to combine the data of those observers whose results are given in detail. The distribution diagram shown in Fig. 8 represents the result of this treatment and shows the combined data of Quinlan and Maré, McKenzie and Phillips, Casida and McKenzie, and the present author. Quinlan and Maré's data are not strictly amenable to treatment in the form of a distribution graph since there is a considerable degree of over-:lapping between the class measures, each observa-:tion being subject to an error of ± 24 hours. This objection applies also, although in much lesser degree, to the other data. Since, however, no one oestrous period has been placed in more than one group the error is not considered sufficient to affect the distribution graph seriously.

The most interesting feature of the combin-:ed distribution graph is its bimodal nature. The main mode at 17 days is followed by a distinct secondary mode at 34-35 days (i.e., double the interval of the chief mode). The periods going to make up the second mode are derived mainly from the data/

data given by Quinlan and Maré but partly also from McKenzie and Phillip's, and Casida and McKenzie's figures. There are clearly two possible explana-:tions of the second mode; it may have resulted from failure on the part of the observers to note an oestrous period or, alternatively, from a failure on the part of the ewe to exhibit the desire to mate at the time when ovulation took place.

Quinlan and Maré's observations were taken at intervals of 24 hours and the ram was not run out with the ewes, so that a proportion of the heat periods of less than 24 hours duration would not be recorded. The same objection is not allowable in the case of McKenzie and Phillip's data and probably also not with Casida and McKenzie's since a "keeled" ram was running almost constantly with the ewes and would almost certainly mark any that came into oestrum. It may be concluded tentatively therefore that the ewe may occasionally show a dioestrous cycle of twice the normal duration, this doubling of the period being accomplished, probably, by the failure of the ewe to show the mating instinct although normal ovulation takes place. The fact that ovulation may take place prior to the first oestrous period of the breeding season lends support to this interpretation.

There are also slight indications of third

and fourth modes around 50 and 67 days. Such modes if they have any real existence might be explained by the occurrence of several successive ovulations unaccompanied by the mating desire, since they represent three and four times the typical period respectively.

The average duration of the oestrous cycle derived from the combined data represented in the distribution graph, but omitting, since the curve is multimodal, all figures above 28 days, is 16.8 days.

There is at present no clear explanation for the few abnormal cycles which do not fall into any of the modal groups. 3.4% of the 697 periods do not fall within 2 days of one or other of the modes. Aberrancies are not confined to any one group of data. Unfortunately in no case is there a record of the cycle lengths before and after the aberrant cycle: it is therefore not possible to say whether any of these cases fall into the same cate-:gory as the fifth and sixth cycles of experimental ewe 816 where the successive discrepancies balance out. Speculation as to the cause of these irregular cycles would be entirely unprofitable since there is no fragment of evidence upon which to base such speculation, but it may be noted that operative interference appears to upset the regularity of the oestrous rhythm (e.g., ewe 710). Drips (50) noted similar irregularities in the oestrous cycle of the white/

white rat after operative interference, but in this case the interference was with the ovaries themselves.

2. THE DURATION OF OESTRUM.

The determination of the duration of heat requires far more careful observation than does the determination of the length of the oestrous cycle and the data in the literature are therefore not nearly so extensive as are those regarding the latter period.

Quinlan and Maré (130) record the durations of 59 oestrous periods in the same number of South African Merino ewes. Figures are also given for two successive periods in nine ewes. Variability between ewes and between successive cycles in the same ewe are indicated. The range of variability shown was from 24 to 96 hours but there was a very strongly marked mode between 36 and 48 hours. The average duration was about 40 hours. In these experiments observations were taken only twice daily, so that each record is subject to an error of ± 12 hours. The data are thus of little value for comparative purposes.

Cole and Miller (41) give data for about one hundred heat periods in 27 grade Rambouillet ewes kept in California. These data are subject to an/

an even greater error than those of Quinlan and Maré, however, since observations were taken only once daily. In a majority of cases the ewes re-:mained on heat for two successive days but about a third of the ewes were on heat for only one day. The range of variation indicated is about 24-72 hours.

McKenzie and Phillips (112) present the most detailed observations that are available in the literature. Their data apply to 97 Hampshire, Shropshire and Southdown ewes kept at Missouri, which were observed through an aggregate total of 247 oestrous periods. The distribution graph given shows an essentially regular distribution round a modal duration of 22-27 hours. The distri-:bution is slightly skew, the classes above the mode being somewhat better represented than those below. This is reflected in the average duration of 26.8 hours. The range of variation was from 5 to 50 hours. The upper limit of variation is considerably below that recorded by other authors. This fact may be regarded as significant since a large number of observations was made. A lower average (19½ hours) and a lower range of variation (8 to 41 hours) was obtained for lambs of the same breeds, but the number of periods observed appears to be only about 20.

The/

The durations of the successive oestrous periods observed in the present experiment are shown in Appendix Table IV. The distribution of the 77 periods which may be compared (that is, those which did not follow operative interference) is shown in Fig. 9. The modal duration for the group is about 28 hours. The distribution is clearly skew, however, and the mean duration is 36 hours. The range of variation shown (3 to 84 hours) is greater than any previously recorded, and as compared with McKenzie and Phillips distribution, the members are not so closely grouped round the mode. The mean deviation of McKenzie and Phillips' data is 11 hours while that shown by the author's data is 22 hours. The great variability shown by the author's animals may be a reflection of the great hetero-:geneity of the group.

The relevant data on the subject of heat duration are summarised in Appendix Tables V and VI.

3. FACTORS WHICH MAY AFFECT THE PERIODICITY AND DURATION OF OESTRUM.

There is an abundance of evidence indicat-:ing that the intensity of ovarian activity may be influenced, in the sheep as in other mammals, both through the genetic constitution and age of the ewe, and through the environment. The capacity for continuous/

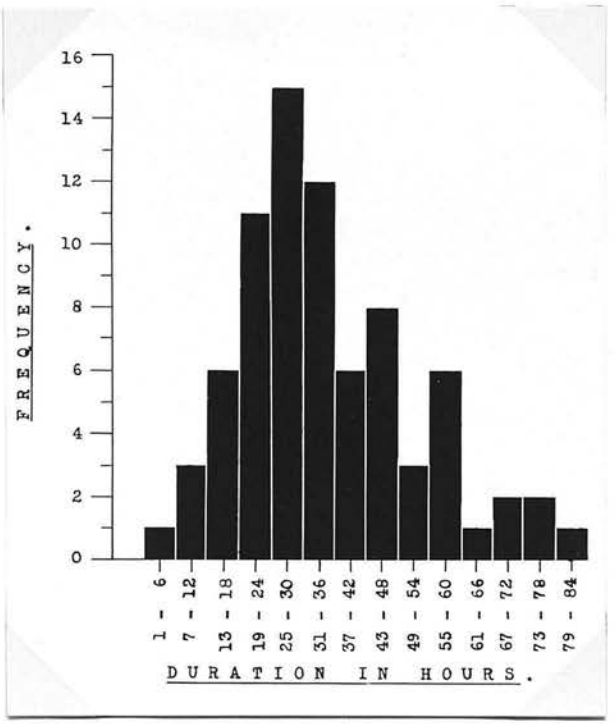


Fig. 9. Duration of Oestrum. Compiled from the author's data and representing 77 periods. Mean 36 hours, mean deviation ± 22 hours.

continuous breeding in the Merino, and the relative-:ly high degree of fertility in the Hampshire Down are breed characters in the strictest sense. It has been shown (129, 14, 77, 32) that the fecundity of a ewe rises gradually until about 5 years of age and thereafter declines slowly. The limitation of the breeding season to certain seasons of the year is in itself adequate evidence of the profound effect of the environment upon ovarian activity.

There are indications, also, that the duration of the phases of the oestrous cycle may be affected through the same media.

A. Breed Differences.

It has already been pointed out that the figures for the modal duration of the oestrous cycle given by Quinlan and Maré and by Darlow and Hawkins for the Merino ewe, are slightly greater than the corresponding figures obtained by other authors for various other breeds of sheep. There may thus be a difference between breeds in the mean duration of the dioestrous cycle, but the number of ewes of other individual breeds examined is insufficient to warrant any conclusion in this matter. McKenzie and Phillips (112) found no significant difference bet-:ween breeds in a group of 97 Hampshire, Southdown and Shropshire ewes. Similarly no breed differences were/

were indicated in the present experiment (Appendix Table III).

On the other hand, such figures as are available for Central European sheep (86, 144, 4) indicate that the cycle in these may be longer than that in the breeds for which accurate data are available (see Appendix Table II). It will not be possible to decide whether breed differences in oestrous periodicity exist until other breeds have been subjected to more precise experimental study.

There is evidence of a more definite nature to show that the duration of oestrum is subject to breed differences. The available figures for individual breeds are shown in Appendix Table VI. The longer heat period in Hampshire ewes as compared with Shropshires and Southdowns is stated by McKenzie and Phillips to be mathematically significant. In the case of the Cheviots only five ewes were involved (21 cycles): the figure is therefore not reliable. The high figure given by Quinlan and Maré for the South African Merino may almost certainly be regarded as significant, since the modal duration is also some 12 hours greater than that indicated by the data given by McKenzie and Phillips and by the data collected in the present investigation for other breeds. A rather long oestrous period is indicated also in the American Rambouillet, which also shows

a/

a high modal duration, but Cole and Miller do not give a figure for the mean duration in this breed.

It has been remarked that the dispersion of the durations of the oestrous periods observed in the present experiment is much greater than that shown by McKenzie and Phillip's data, while the range of variation is greater than any previously observed. This greater variability may probably be ascribed to the greater heterogeneity of the author's animals with regard to genetic constitution.

It may not be said, however, that the differences observed between the various records are certainly due to breed differences: the possibility that the variations noted are due to methods of management or to climatic differences cannot be eliminated. Between the three breeds observed by McKenzie and Phillips there was relatively little difference in the duration of heat: this might be ascribed either to the similarity of the three breeds or to the uniform conditions of management and climate.

B. Age.

The oestrous period of the lamb is significantly shorter than that of the yearling and older ewe, according to the results of McKenzie and Phillips, for Hampshires and Shropshires. The average/

average duration of heat in lambs was 8½ hours less than that for yearlings and older ewes in both breeds. However, only 7 Hampshire and 13 Shrop-:shire lambs appear to have been examined, and only the first heat period in each seems to have been observed.

The modal duration for lambs is not given but the observed difference between ewes and lambs appears to be due mainly to the absence of particular-:ly long heat periods. Lambs did not differ from older ewes in the periodicity of heat and eighteen month old ewes did not differ from older animals in either periodicity or duration of the heat period.

Of the ewes included in Appendix Tables III and IV; 773 and 751 were yearlings (18 months old virgins), the remainder had all raised one or more crops of lambs. No difference is apparent between the yearlings and the older ewes in either duration or periodicity of oestrum.

In the cow the duration of the oestrous cycle and of the heat period both tend to increase slightly with age (Hammond (66)).

C. Environmental and Climatic Influences.

Marshall (101, 106) has stated that the dioestrous cycle in Scottish Blackfaced sheep under Highland conditions lasts three weeks and the heat period/

period up to five days, but that under lowland conditions the dioestrous cycle is about 16 days and the oestrous period only about 2 days in length. The number of oestrous periods in the breeding season is also said to be increased under lowland conditions - "where the number of recurrent oestrous periods is increased [by the change from highland to lowland conditions] the duration of oestrous is shorter, while, whether partly or entirely as a consequence of this latter fact, the length of the dioestrous cycle is also less."

From figures given by Alongi (4) and by Sanctis (141) it would appear that the oestrous cycle of sheep in Sicily is considerably shorter than that found in Italy itself (Umbria district). The figures given by Alongi for the two areas are 15 and 20 days respectively. This difference is attributed to the climatic difference since the mean annual temperatures for the two areas differ by $4^{o}C$. The supposed temperature effect was found in the sheep and goat only: in the sow, bitch and cow there were no appreciably differences, in the mare the effect was reversed, the cycle being 22 days in length in Sicily and 15 days in Umbria. It is not clear whether the sheep used in the two areas were of the same breed. In the absence of further data it seems advisable to attribute the observed differences/

differences to genetic influences since the exact figures available for more northerly zones approach the figure given for Sicily rather than that for Umbria. It may be noted, however, that all the data available for Central European areas indicate cycles approaching 21 days in length (86,144,141,4) However, the native sheep of Iceland (O.Borealis) have a dioestrous cycle 14 to 16 days in length (164).

In the cow it has been shown by Hammond (66) that the duration of the dioestrous cycle and the length of the heat period are both greater in summer than in winter. Lee (91) has shown that in the rat exposure to cold lengthens the dioestrous cycle considerably: this change was thought to be effected through the general metabolic level of the rats since body temperature and activity are both lowered under cold conditions.

While direct observations on hill sheep have not been made, the concensus of opinion among breeders with whom the author has communicated does not indicate a significantly different periodicity or duration of oestrum from that observed in low-ground sheep. It should be pointed out that Marshall's observations on this subject are not based on direct observation.

While the number of animals studied is too small/

small to justify a general conclusion, Appendix Tables III and IV do not indicate any marked trend of alteration in the duration or periodicity of oestrum during the breeding season, which, in the extreme instances, extends over a period of six months and covers a considerable range of climatic conditions.

Appendix Table IV indicates a quite defin-:ite tendency for the first oestrous period of the breeding season to be shorter than the succeeding periods: the first heat was also, in general, less intense than the following ones. It has been shown, moreover, that the first heat period is preceded by one or more cycles of ovulation which are unaccompanied by the desire to mate. It would be unreasonable to ascribe these differences primarily to seasonal influences however.

From about the end of December onwards the intensity of heat displayed by the ewes appeared to decrease steadily, but since the intensity of heat could be measured only by the mutual behaviour of the ram and ewe, it is probable that the apparent falling off in intensity of heat was in reality due to a decrease in the sexual energy of the ram.

Appendix Table III suggests a tendency for the cycle length to increase slightly during the course/

course of the breeding season,* but no similar trend is discernible in the more extensive data recorded in Quinlan and Maré's Table X, which covers a period of eight months.

It has already been mentioned that the modal duration of the oestrous cycle shown by Quinlan and Maré's data is somewhat greater than that observed by other authors: this difference, however, may be explained as a breed difference rather than as a difference due to the different climatic conditions of South Africa since Darlow and Hawkins obtained a similar high figure for Merino ewes in North America.

Benedict and Ritzman (19) have shown that the energy exchange of the sheep is practically un-:affected by a fall in environmental temperature, but temperatures above the normal produced disturbances in metabolism. These findings might have been anticipated since the heavy fleece of the sheep pro-:vides a very effective barrier against heat loss, and very little water is lost through the skin. Under conditions of abnormally high temperature the type of respiration is changed. Rainfall is much more likely to influence metabolism than temperature changes since the heat loss through a wet fleece is probably much greater than that through a dry fleece.

D. Nutrition./

*An interval of six weeks separated the first heat periods of the first and last ewes to come into heat; this is not indicated in the Table.

D. Nutrition.

Nutrition is known to affect the oestrous cycle in several animals. Hammond (66) has shown that the cycle and heat period are both curtailed in fat cattle. In guinea pigs (Papanicalaou and Stockard (123)), and rats (Long and Evans (95)) the interovulation period is increased by under feeding. Shiraki (148) found that the dioestrous period was increased in rats fattened on brain and cholestrin. Lecithin, on the other hand caused sexual stimulation and increased the duration of heat. While many authorities claim that rich feeding ("flushing") stimulates sexual activity in the ewe, no observations upon the effect of nutrition upon the duration or intensity of oestrum or upon its periodicity have been made. An obese condition is held to be a frequent cause of infertility in ewes and is said to lead to lessened ovarian activity and persistence of the corpus luteum spurium (Quinlan and Maré (130)). There appears to be no adequate foundation for the last statement in the case of sheep however. Loeb (94) Papanicalaou and Stockard (123) and others, have shown that follicular atrophy follows underfeeding of rodents.

Of the ewes included in Appendix Tables III and IV, 705, 726 and 710 were markedly leaner than the average and 631 was fat. No difference is discernible/

discernible between these ewes and the others included in the tables.

E. Sterile Service and Proximity of the Male.

It is well known that the phenomenon of pseudopregnancy, which occurs without service in the bitch, may be brought about in the rat, guinea-pig, etc. by sterile service or by cervical stimulation. Apparently authentic instances of pseudopregnancy in the sow and cow, probably as a result of sterile service, have been recorded, but the phenomenon is unknown in the sheep. The treatment of the experimental ewes recorded in Appendix Tables III and IV with regard to service by the vasectomised ram differed considerably: at the one extreme the ewe was kept away from the ram during an entire oestrous cycle, while at the other extreme the ram was run out with the ewe and allowed to serve her as often as he wished (about every half hour during heat.) None of the differences shown in the Tables could be related in any way to these differences in treatment. All these ewes were used regularly for the taking of vaginal smears, however, and the possibility still existed that stimulation in this way might affect the oestrous cycle. A check on this possibility was effected by observing ten other ewes not used for smearing and kept away from the ram, the progress of the/

of the oestrous cycles being determined by the behaviour of the ewes and the changes in the external genitalia. The length of the oestrous period could not be determined accurately in this way since oestrous symptoms usually disappear long before willingness to mate, but the length of the dioestrous cycle was readily determined. Twelve cycles and 14 oestrous periods observed in this way all fell well within the range of variability shown by the experimental ewes.

Further evidence that the use of a vasectomised ram for the determination of the onset of heat does not affect the cycle periodicity may be derived from sheep-breeding practice. When a ram is put with a flock of ewes at the height of the breeding season seventeen days are allowed for the ram to serve all the ewes: observations on the flocks at the Institute of Animal Genetics have shown that service of the ewes is spread fairly evenly over the first sixteen days after the ram is put out.

F. Removal of the Corpus Luteum.

Many observations are on record which show that the removal of the spurious corpus luteum cuts short the dioestrous period and allows the animal to come back into heat much sooner than would ordinarily be the case (Hammond (66) in the cow, Loeb (93) and/

and Papanicolaou and Stockard (123) in the guinea-pig, etc.). Hammond also found that the duration of the heat period following experimental removal of the corpus luteum is considerably below the average. This fact is in accordance with his other observation, that the length of the oestrous period in the intact animal is positively correlated to the duration of the previous oestrous cycle.

A single operation for the removal of the corpus luteum was performed on ewe 704. The corpus was removed on the 2nd day of the sixth successive cycle. The length of the dioestrous period and of the subsequent oestrous period were both raised somewhat, the former to 19¾ days and the latter to 64 hours. At the time of operation it was noted that there were only rather small follicles in the ovaries. The heat period following removal of the corpus luteum terminated the breeding season of that particular ewe.

In two other ewes (726 and 710) caeserian section was performed for the removal of the foetus on the 22nd and 16th days following insemination respectively. In the case of ewe 726 the corpus luteum was left intact. This ewe came into heat 10 days after the operation. In the case of ewe 710 the corpora lutea were removed as well as the foetus. Oestrum in this ewe returned after 2½ days. In both cases the heat periods immediately following the operation/

operation were of normal duration. Both these ewes showed fair sized follicles in the ovaries at the time of operation.

These three experiments are by no means conclusive but they indicate that the sheep probably agrees with other animals in that removal of the corpus luteum hastens the onset of a new oestrous period.

G. Individual Differences.

Differences between one individual and another which could not be correlated with breed, age or condition of the ewe, in respect to duration and intensity of heat, were recorded in the present experiment. Ewe 701 in particular showed consistent-:ly long heat periods (see Appendix Table IV) and this was associated with particularly intense sexual desire, as measured by her behaviour towards the ram. Heat in ewe 714 also tended to be more intense than usual while in ewe 631 heat was so slight that some difficulty was experienced in determining its limits. Individual differences in the intensity of sexual desire are well recognised by sheep breeders. How-:ever, intensity of heat is a characteristic which is very difficult to assess since it is uncertain to what extent it is dependent upon the reaction of the ewe to experimental conditions. Ewe 631 was a particularly nervous animal.

No individual differances are discernible in the duration of the dioestrous cycle, either in the author's data (Appendix Table III) or in those given by Quinlan and Maré.

III : THE INTERRELATIONSHIP BETWEEN DURATION, INTENSITY AND PERIODICITY OF OESTRUM.

It has already been noted that in ewe 701 long heat periods were associated with high intensity of sexual desire. While the intensity of heat was too vague a characteristic to permit direct comparison the same rule seemed to hold generally: long heat periods of all ewes tended to be rather more intense than short periods. The first heat period of the breeding season was both shorter and less intense than the following periods. Weber (166) found that cows which showed intense heat had, on the average, longer heat periods.

Hammond (66) has shown that there is a distinct positive correlation between the length of the oestrous cycle and the duration of the subsequent oestrum in cattle, while if the cycle is cut short by removal of the corpus luteum, the duration of the succeeding heat period is correspondingly short. Marshall (101) maintains that a general relationship between the duration of heat and the duration of the oestrous cycle holds with sheep (see page 110).

Unfortunately, previous authors have not considered the possible relationship between the duration of the heat period and the duration of the preceding and subsequent cycles in the sheep; nor are there published records from which data can be extracted./

extracted. The data derived from the present experiments are summarised in Tables III, IV and V.

Table III shows clearly that there is no relation between the duration of heat and the duration of the preceding cycle, nor is there a correlation with the duration of the subsequent cycle, of which the heat period forms a part. On the other hand, from Table IV it appears that there is a very definite negative correlation between the duration of oestrum and the duration of both preceding and subsequent interoestrous periods (i.e., the interval between the end of one heat period and the beginning of the next). It is a little difficult to decide whether both these correlations really exist since the distribution of the oestrous periods is by no means random: an examination of those cases where two successive heat periods differ greatly in duration indicates, however, that both correlations have a real existence. These correlations, in the absence of any correlation between duration of heat and duartion of the cycle as a whole, indicate that the oestrous period is able to vary independently within the body of a relatively constant oestrous cycle, and since a negative correlation to both preceding and succeeding interoestrous periods exists the oestrous period, may, apparently, "expand" into both preceding and succeeding cycles.

The/

TABLE III.

Relation between Duration of Oestrum and Durations of Preceding and Subsequent Dioestrous Cycles.

Oestrum. (Hours)	Preceding Cycle. (Days)	Average.	Subsequent Cycle. (Days)	Average.
1 - 10	18½.	(18.5)		
11 - 20	18½, 17½, 16, 17¾.	17.44	15, 16¾, 16½, 16¼, 15¾, 17, 15½, 16.	16.09
21 - 30	17¼, 15¾, 15, 16, 16¾, 16¾, 16¾, 18½, 16½, 16½, 16¼, 17¼, 15¾, 17, 15¾, 17, 16½, 16½ 16, 16¼, 17.	16.52	15¾, 18½, 16, 16¾, 16½, 16¾, 16¼, 16¼, 16½, 16½, 16½, 17, 15¾, 17½, 18½, 16½, 16½, 16, 16¼, 17, 17¾.	16.71
31 - 40	16½, 16½, 16¾, 17¼, 17½, 16¾, 17¼, 16½, 15¾, 16¾, 15½, 15½, 16¼.	16.52	16½, 16½, 16¾, 16¾, 16¾, 17½, 16¾, 17¼, 18½, 16½, 16¾, 17¼, 15¼, 15¼, 16¼, 17.	16.72
41 - 50	16, 16½, 16¾, 16¼, 16¼, 16¾, 15½.	16.30	15¾, 17, 16½, 16¾, 17¼, 16½, 15½.	16.46
51 - 60	15, 15¾, 17, 15½, 17¼, 17½, 16½, 16½, 15¼.	16.36	16, 16, 17¼, 17¼, 17½, 17, 15¾.	16.68
61 - 70	17, 15¼.	16.12	17½.	(17.50)
71 - 80	16, 16¾.	16.37	15, 16¾.	15.87
81 - 90	17½.	(17.50)		

TABLE IV.

Relation between Duration of Oestrum and Durations of Preceding and Subsequent Interoestrous Periods.

Oestrum. (Hours)	Preceding Interoestrum. (Days)	Average.	Subsequent Interoestrum. (Days)	Average.
0 - 10	17¼.	(17.25)		
11 - 20	17½, 16½, 15, 16½.	16.37	14¼, 16¼, 16¾, 15¾, 15¼, 16½, 14¾, 15¼.	15.47
21 - 30	15, 14¾, 14¼, 15, 15¾, 16¼, 15½, 17, 15¼, 15½, 15¾, 15½ 15¼, 15¾, 14½, 16½, 15½, 15½ 15¼, 15¼, 15¾.	15.46	14¾, 17½, 15, 15¾, 15½, 15¾, 15¼, 15, 15¼, 15¼, 15½, 15¾, 14½, 16½, 17¼, 15½, 15½, 15, 15¼, 16, 16½.	15.63
31 - 40	15½, 15¾, 15¼, 15¼, 16, 15¼, 15¾, 15¼, 13¼, 15¼, 14¾, 13¾, 14½,	15.04	15, 14¾, 15, 15¼, 15½, 16, 15¼, 15¾, 17, 15, 15¼, 15½, 14, 13¾, 14½, 15¾.	15.20
41 - 50	13½, 15, 15¾, 15¼, 15, 15, 15½,	15.00	13¾, 15¼, 14½, 15, 15¼, 14½, 13¾.	14.57
51 - 60	11¾, 13¾, 15¼, 14¾, 14¾, 15¼, 14½, 14½, 14.	14.28	13½, 14, 15, 14¾, 15¼, 14¾, 13¼.	14.36
61 - 70	14¾, 13¾.	14.25	14½.	(14.50)
71 - 80	14, 13½.	13.75	11¾, 13½.	12.62
81 - 90	14½.	(14.50)		

The duration of the interoestrous period is much more variable than the duration of the whole cycle, since it is subject not only to the variations in the duration of the cycle but also to those of oestrum alone, which are considerable. (Compare Figs. 6 and 7, and Appendix Tables III and VII.) The dispersion of the dioestrous cycles, as measured by the mean deviation from the mean, is 19.3 hours (4.8%) while that of interoestrum is 21.9 hours (6.0%).

If the oestrous period may "expand" into both preceding and succeeding interoestrous periods then it follows that the cycle length, measured from some point within the oestrous periods should be more constant than the cycle length measured from beginn-:ing to beginning or from end to end of the successive periods. Table V shows the average length and dispersion of all the oestrous cycles observed accurately in the present experiment, the cycles being measured from three different loci. It will be seen that the mid-oestrum to mid-oestrum measurement is slightly less variable than the commencement to commencement and the end to end measurements while the end to end measurement is the most variable. This might perhaps be taken as an indication that the end of heat is the least rigidly determined part of the period, but the significance of/

TABLE V.

Mean Duration of Oestrous Cycle Measured from Different Loci.

Commencement of Heat to Commencement of Heat.		Mid-Heat to Mid-Heat.		End of Heat to End of Heat.	
Mean Duration	Mean Deviation	Mean Duration	Mean Deviation	Mean Duration	Mean Deviation
16.70 days	± 19.286 hours	16.71 days	± 19.236 hours	16.71 days	± 19.661 hours
Mean Duration of Interoestrum 15.02 days. Mean Deviation ± 21.93 hours.					

of the differences between the three measurements is doubtful.

If the lability of the commencement of heat accounted in any marked degree for the variability in the duration of heat then an individual long heat period would tend to shorten the previous cycle, measured from commencement to commencement of heat, and to lengthen the following cycle; in other words the duration of heat would be correlated negatively to the previous and positively to the succeeding oestrous cycles. It has already been noted that such is not the case: no correlation at all exists between the duration of heat and that of the preceding or following dioestrous cycles.

These interrelations between the durations of the phases of the oestrous cycle might be inter-:preted in the following manner. There is a fundamental rhythm in oestrous periodicity which is subject to only very slight variations: it will be shown that the immediate regulator of this rhythm is probably the duration of life of the corpus luteum, although the factors which control this are as yet unknown. Oestrum is closely associated with the development of Graafian follicles and development of these takes place only when there are no active corpora lutea in the ovaries. The onset of heat is thus determined by the degeneration of the corpus luteum of the previous cycle. During the maturation of/

of the Graafian follicles the ovarian follicular hormone is produced. The amount produced varies considerably, however; at the first one or two periods of follicular development in the breeding season the amount is insufficient to produce heat, although the secondary changes in the vagina are produced. During the intense heat periods at the height of the breeding season, on the other hand, the hormone is produced abundantly and its effects per-:sist for some time after ovulation has taken place. Thus the end of heat is subject to considerable variation. The onset of heat, depending directly upon the maturation of the follicle is subject to only slight variation, since development of the follicle is very rapid when the corpus luteum once begins to degenerate. Since the duration of heat, on this hypothesis, is regulated simply by the amount of heat-producing substance produced, and consequently upon the time taken to reduce the concentration of this substance in the blood below the threshold necessary for the elicitation of heat by excretion through the kidneys, the duration of heat does not affect the rhythm of the cycle.

This interpretation seems to agree with the finding (see page 132) that heat comes on very suddenly but passes off very slowly.

IV : THE EXTERNAL MANIFESTATIONS OF THE OESTROUS CYCLE.

The sexual phase or period of "heat" in the ewe is much less clearly demarcated from the rest of the cycle by differences in behaviour and in the appearance of the external genital organs than is the case in the sow and the cow. In the cow, for example, the mating period is marked, in the absence of the male, by general restlessness, mounting of other females or the permitting of mounting by other females, and by constant lowing. Similar changes occur in the sow (111). Marked differences in the behaviour of the ewe do not, as a rule, occur in the complete absence of males: she may feed less than usual and sometimes stands with her head lowered for a time. Occasionally she may show a tendency to separate from the flock. On hill farms it is quite a common occurrence for a ewe to leave a flock and travel for several miles in search of a ram. How-:ever, all these changes in behaviour occur sporadi-:cally in their milder forms and none of them is of the slightest diagnostic value. If, on the other hand, a ram is near although not actually running with the ewes, then there may be distinct changes in the behaviour of the ewes coming on heat. Under these conditions a ewe will frequently cease feeding altogether/

altogether and leave the flock when she comes on heat, taking up a position as near to the ram as possible, and she will occasionally exchange bleats with him. These changes in behaviour occur only during the long, intense heat periods, that are experienced at the height of the breeding season, however: it is rarely that the more slight heat periods are accompanied by any change in the behaviour of the ewe at all, beyond that of permitting service by the ram.

Changes of a more definite nature are observable in the external genital organs of the ewe. During pro-oestrum and early oestrum - the two phases are inseparable in the ewe - the external labia of the vulva are usually swollen and congested with blood: this congestion often spreads on to the surrounding skin and sometimes the whole tail region is involved. As a rule, congestion appears rather suddenly about an hour or less before the onset of receptivity, and dies away gradually before the end of the heat period if the ewe is not mated. There is however, considerable variation: congestion may fail to occur at all throughout the heat period, or it may persist well into metoestrum. At the peak of the heat period the vulva is moist and there is sometimes a slight continuous excretion of mucus. More usually the mucus is expelled forcibly and in quantity, usually at the time of urination.

During/

During the metoestrum and dioestrum a yellow, pus-like admixture of mucus and cells is excreted from the vulva: some of this material usually adheres to the tail and the lips of the vulva, where it dries and changes to a deep red-brown colour. The presence of the fresh yellow excretion is a fair indication of the late metoestrous phase, but the converse is by no means true.

Wiltshire (cit. Marshall (101)) and others have recorded the excretion of blood from the vulva of the sheep during pro-oestrum: this was not observed at any time in any of the experimental ewes observed by the present writer nor were erythrocytes found in the vaginal smears from these animals. About 30 samples of vaginal contents were tested by means of the benzidine test to determine whether small quantities of blood were present in a laked condition, but in no case was a definitely positive result obtained. Blood was found in the vaginae of many of the ewes examined at the slaughter house but in only very few cases did this blood appear to be of physiological origin: in the remainder it could almost certainly be ascribed to contamination by the butchers. In all cases where blood of natural origin was found, it was found in metoestrum, not in pro-oestrum.

Thus it appears that blood is rarely/in the present vagina/

vagina during pro-oestrum, if it occurs at all. It seems probable that, where isolated observations were made, the red-brown, dried excretion, which usually encrusts the tail in dioestrum, has been mistaken for dried blood.

Contractions of the vulva of a receptive nature, tending to draw in the stimulating object can be induced by rubbing the external labia gently at all phases of the oestrous cycle, but the con-:tractions are strongest and the sensitivity of the vulva is greatest during oestrum.

Many sheep breeders contend that the approach of the breeding season is heralded by a "rusty" discoloration of the wool on and around the tail. While the present author has observed this discoloration, no observations have been made to determine its relation to sexual activity. There is clearly no primary reason to doubt the truth of the contention for substances excreted in the urine dur-:ing the sex cycle might quite easily give rise to coloured decomposition products.

The one criterion that can be regarded as of absolute diagnostic value in the detection of the mating period lies in the mutual behaviour of the ewe and ram. During the heat period the ewe stands still when the ram attempts to serve her; the tail is lifted or vibrated to allow the ram to sniff at the/

the vulva. If the heat period is an intense one the ewe will "pet" the ram both before and after service, licking his face and rubbing her head against his side. An active ram will attempt to serve a ewe whether she is on heat or not but at the time of oestrus his efforts are much more intense and, if necessary, prolonged. It is the common experience of farmers that a ram will inflict serious injuries to his head, sometimes so serious as to necessitate destruction, in his efforts to butt down an obstruction intervening between himself and a recep-:tive ewe, although he may be separated from her by a distance of 200 yards ~~years~~ or more.

If a ram approaches a non-receptive ewe, a very characteristic series of events occurs. Gener-:ally, the ewe does not move away immediately but first micturates and then moves off. The ram smells the urine carefully, then raises his head and sniffs the air perhaps for several minutes. This careful scenting of the urine and air is accompanied by a curling up of the upper lip, so that the teeth are exposed. Deliberate scenting of the air in this manner seems to appear only during the search for a receptive ewe: it has not been observed except after the scenting of urine.

The commencement of the heat period is sharply demarcated: full receptivity is usually reached/

reached within 30 to 60 minutes after the time when the ram begins to display more interest than usual in the ewe. Rarely, however, the ram will "hang around" a ewe for several hours before she becomes receptive, as is often the case with cattle (Hammond (66)). The end of heat, on the other hand is much less sudden: receptivity shows a gradual decline over a period of several hours, and the decision as to whether a ewe is receptive or not frequently depends on the degree of persistency shown by the ram. The finding of Quinlan and Maré (130), that oestrous comes on gradually over a period of several hours, has not been confirmed.

V : CYCLICAL CHANGES IN THE OVARIES

1. THE STRUCTURE OF THE OVARIES.

The ovaries of the ewe are compact white bodies about 2 cms. long and more or less bean-shaped in form. Each ovary weighs, on the average, about 1.5 grams. Along the length of one side the ovary is attached to part of the broad ligament and to a portion of the fimbriated funnel of the Fallopian tube. Otherwise, it hangs loose in the abdominal cavity: the ampulla of the Fallopian tube does not surround the ovary.

The central core of the ovary and the part which is near to the attachment are composed of dense connective tissue, which contains a great number of large blood vessels. The peripheral parts are also largely composed of connective tissue but in addition there are many Graafian follicles and corpora lutea in various stages of development. The surface of the ovary is smooth since it is covered by a thin tunica albuginea and a germinal epithelium.

2. GROSS CHANGES IN THE OVARIES.

An outline of the anatomical changes in the ovaries during the dioestrous cycle has already been given./

given. In essence, the changes during each cycle consist of the growth and maturation of a crop of Graafian follicles, the bursting of these follicles and the development from each of them of a corpus luteum, and the subsequent degeneration of these corpora.

The cyclical changes in the ovaries have been followed mainly from slaughter house material: the ewes killed at the Institute provided a useful check on the conclusions arrived at in this way. It was found that all the genitalia examined, with the exception of a very few abnormalities, could be classified according to the stage of reproductive activity by superficial examination of the ovaries and uterus. All the material examined was relegated to one or another of eight classes. Classes I. and II. consisted of immature lambs and ewes killed in anoestrum, the ovaries contained small follicles up to 3 mm. in diameter (I.) or moderately large follic-:les up to 6 mm. (II.) but no corpora lutea. Classes III. to VII. consisted of genitalia from ewes in stages of the dioestrous cycle: the ovaries contain-:ed either ripe Graafian follicles or corpora lutea in successive stages of development or regression. Class VIII. consisted of genitalia from pregnant ewes. While there was inevitably a good deal of overlapping between the classes, little difficulty was/

was experienced in arranging the material from ewes killed during the dioestrous cycle in the five groups.

The sample of genitalia examined on each occasion that the abattoir was visited consisted of all the genitalia removed from ewes slaughtered during the time that the author was present in the slaughter house. There is thus every reason to believe that the samples collected were completely "random". This being the case, the proportion of the ewes in Classes III. to VII. relegated to any one of the Classes constitutes an indication of the relative duration of the phase of the cycle which that Class represents. The mean duration of the dioestrous cycle being known to be 16.4 days, it has been possible to convert the relative durations into actual values. The relevant data are set out in Table VI.

TABLE VI.

Duration of the Five Ovarian Phases.

Class	Total No. of Genitalia. (A)	A x $\frac{16.4}{648}$ Duration in Days.	Phase of Cycle.
III	34	0.9	1st - 2nd day
IV	81	2.0	2nd - 4th "
V	132	3.3	5th - 7th "
VI	311	7.9	8th -16th "
VII	90	2.3	16th - 1st "
Totals	648	16.4	

The dividing line between Classes III. and IV. was ovulation: although there is no doubt considerable variation in the time of ovulation, this appears to take place usually about 30 hours after the commencement of heat, i.e., during the second day of the cycle. The phases of the cycle represented by each of the Classes on this basis are indicated in the last column of the Table.

Apart from the changes in the corpora lutea and the Graafian follicles, the only anatomical changes in the ovary are changes in vascularity. During oestrum, the ovaries, in common with the rest of the generative tract, are congested. There are, of course, considerable changes in the size and weight of the ovaries but these are all referable to the development of follicles and corpora lutea. The weight of the ovary is naturally greatest when one or more active corpora lutea are present.

The growth of the follicle and of the corpus luteum has been followed by measurement of these bodies in fixed material: since the fresh follicle immediately collapses on puncture it was impossible to take measurements on fresh material. No doubt the fresh follicle is somewhat larger than its fixed counterpart since there are always signs of shrinkage in the fixed follicle even when the most carefully balanced fixatives are used. Measurements of/

of the diameters of the corpora lutea and of the largest follicles were made for a total of 195 pairs of ovaries. Gross sections were cut through the centre of each body and the diameters recorded were the means of two measurements, the greatest diameter and the diameter at right angles to this.

A great many sketches of the appearance of sagittal sections through ovaries were made for record purposes when portions of the ovaries were embedded in paraffin for histological examination: a number of these sketches have been arranged in series and reproduced in Figs. 10 to 13. It has been found that the fixed ovary possesses insuffic-:ient optical differentiation to allow it to be photographed satisfactorily. Coloured drawings of whole ovaries and of sections through ovaries of ewes have been published by Küpfer (86 supplement) and by Quinlan and Maré (130). While the present author is unable to agree entirely with the conclusions of these investigators, the ovarian changes figured are essentially the same as those found in the present investigation.

Photographs showing the appearance of the fresh corpus luteum are given in Figs. 14 and 15.

A. <u>The Corpus Luteum</u>.

The development of the corpus luteum commences/

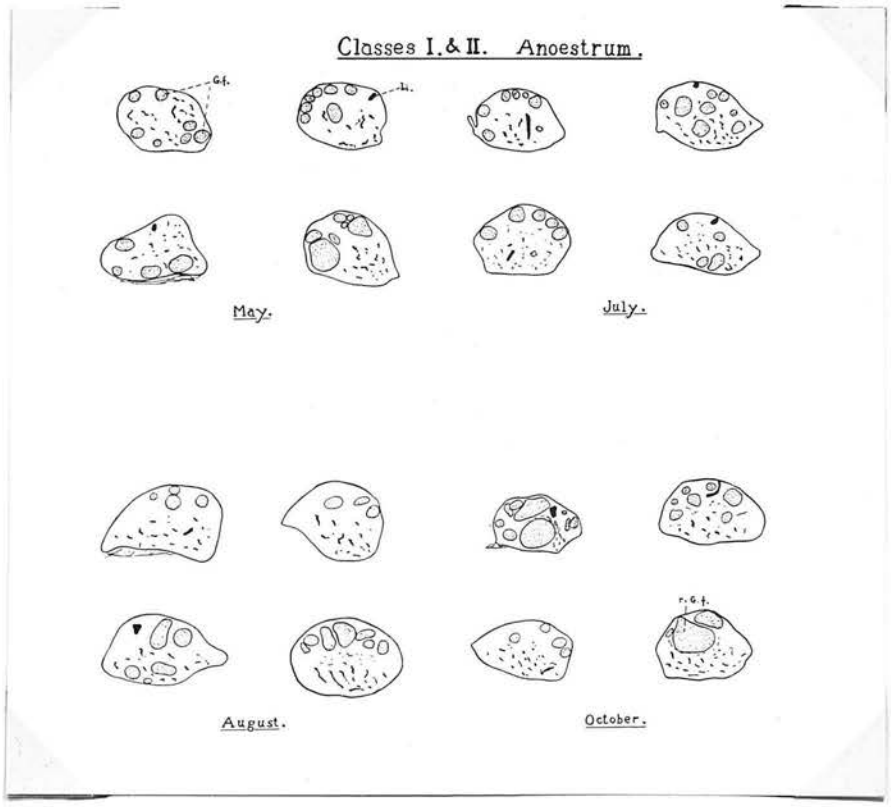


Fig. 10. Sections through ovaries of ewes killed during successive stages of anoestrum. (Diagrammatic). About half natural size.

G.f. Graafian follicles.
r.G.f. ripe Graafian follicle.
li. lipochrome patch, i.e., remains of an old corpus luteum.

(Identical with Fig. 4.)

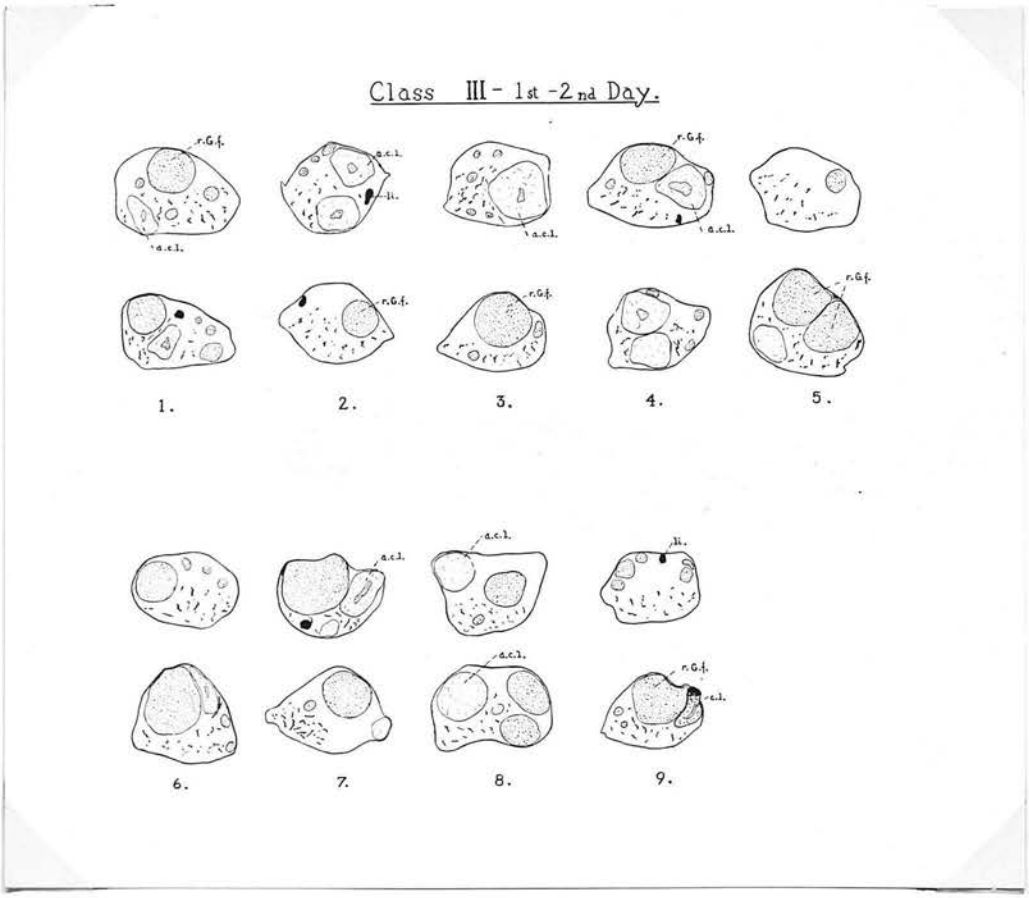


Fig. 11. Sections through ovaries of ewes killed during the first 24 hours of oestrum. (Diagrammatic). About half natural size.

r.G.f.	ripe Graafian follicles.
c.l.	corpus luteum.
a.c.l.	atrophic corpora lutea.
li.	lipochrome patches, i.e., remains of former corpora lutea.

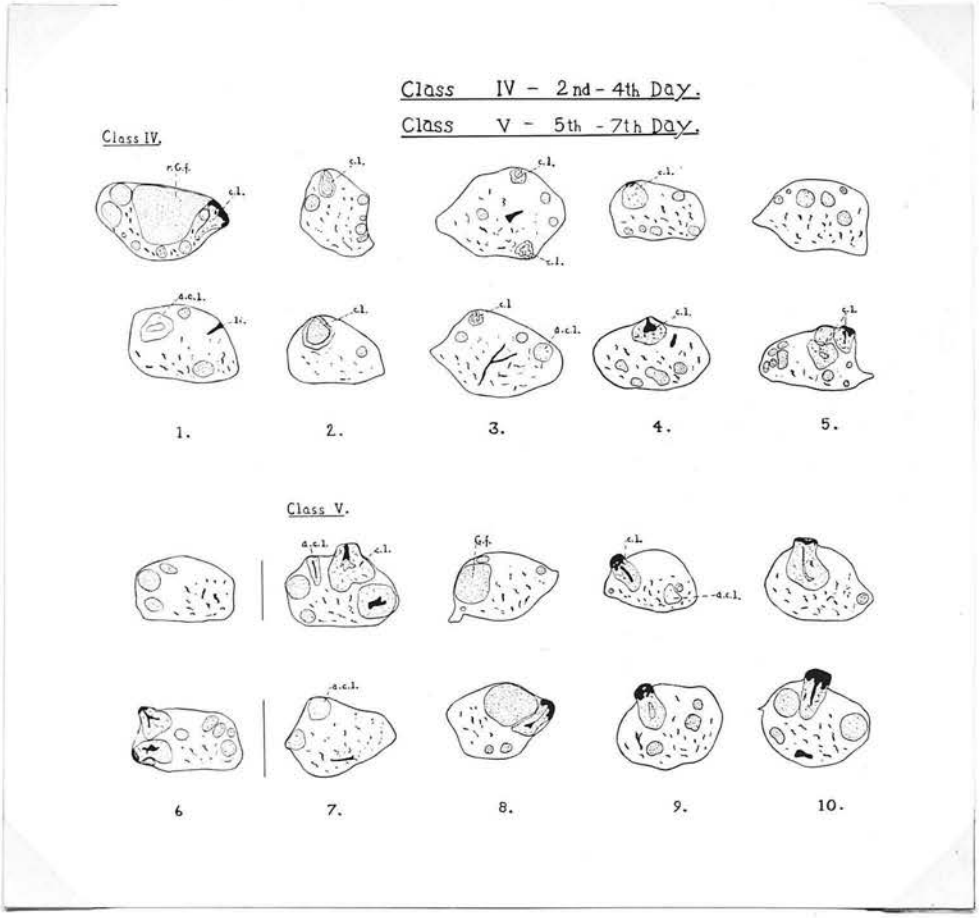


Fig. 12. Sections through ovaries of ewes killed during late oestrum and met-:oestrum. (Diagrammatic) About half natural size.

G.f.	Graafian follicle.
r.G.f.	ripe Graafian follicle.
c.l.	corpora lutea.
a.c.l	atrophic corpora lutea.
li.	lipochrome patch, i.e., remains of a former corpus luteum.

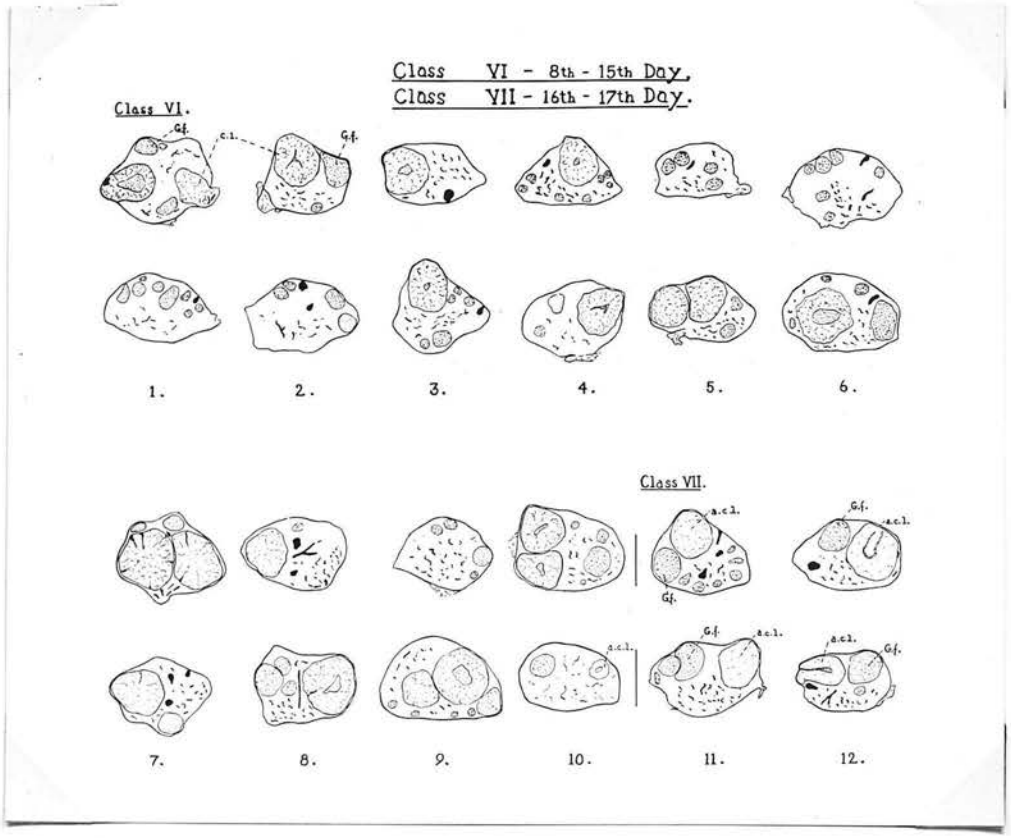


Fig. 13. Sections through ovaries of ewes killed during late metoestrum, di-:oestrum and pro-oestrum. (Diagrammatic) About half natural size.

G.f. Graafian follicles.
c.l. corpora lutea.
a.c.l. atrophic corpora lutea.

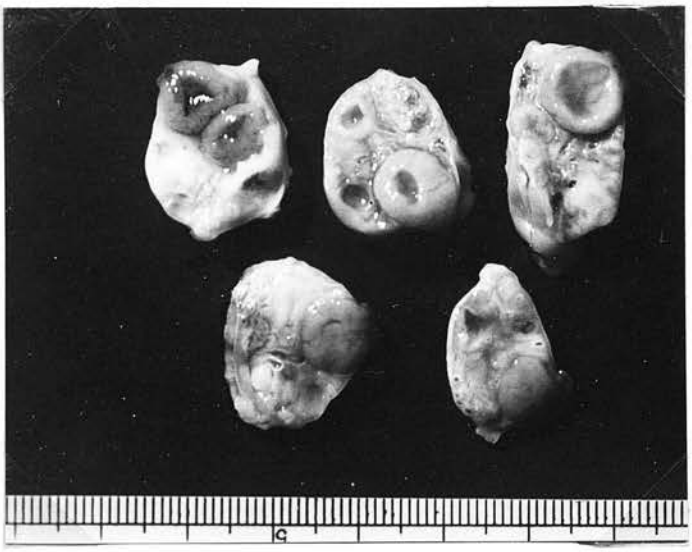


Fig. 14. Sections through fresh ovaries of Class V. ewes.

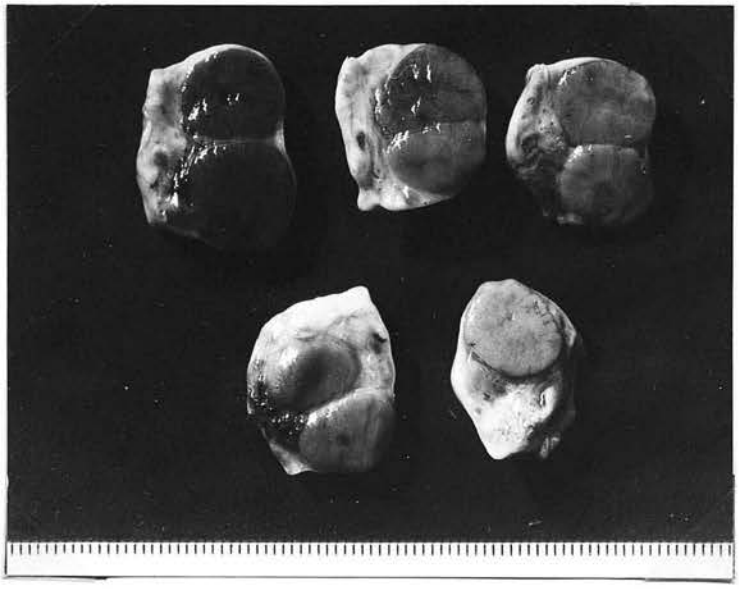


Fig. 15. Sections through fresh ovaries of Class VI. ewes.

commences immediately after the rupture of the Graafian follicle. Usually, the follicle does not collapse completely, a small amount of fluid being retained in the cavity of the young corpus luteum. In most mammals, the collapse of the follicle is associated with a small amount of haemorrhage but in the ewe haemorrhage rarely takes place. The very young corpus luteum is represented on the surface of the ovary by a minute red spot, usually less than a millimetre in diameter. As development proceeds the corpus luteum begins to project from the ovary in the form of a deep flesh-red coloured, rosette-shaped prominence, which ultimately reaches a diameter of about 5 to 7 millimetres. This prominence is formed by an overgrowth of luteal cells protruding through the point of rupture of the tunica albuginea. Its deep red colour is due to the occurrence of haemorrhage in this part of the corpus but there is rarely any bleeding from the surface. There is always a shallow depression in the centre of the prominence. When the corpus luteum approaches its maximum development the tunica albuginea commences to grow over the protruding luteal cells and the corpus appears to gradually sink into the ovary, the latter again assuming a smooth outline. In the later stages of its development the corpus luteum may be seen through the tunica as a deep red body and as a rule a number of large blood vessels develop on the outer/

outer surface of the corpus luteum and can be seen readily.

When atrophy sets in the red colour rapidly disappears and the regressing corpus is represented superficially by a hard white or faintly yellow area of the ovary in which there are no Graafian follicles: sometimes the corpus continues to protrude slightly from the ovary.

Ovulation takes place during the second day of the cycle. This marks the commencement of Stage IV. Development of the corpus luteum soon begins and the protrusion of cells from the point of rupture commences about 48 hours later (commencement of Stage V.). Full development of the corpus luteum is reached at about the 8th day when the corpus becomes covered in by the tunica albuginea (commencement of Stage VI.). Atrophy commences about 24-48 hours before the onset of the next heat period. According to Quinlan and Maré, the corpus luteum of the previous cycle remains "little changed either in size or colour at the commencement of oestrous". This has not been found to be the case in the present investigation. The ewes killed at the Institute before the onset of heat or during the first few hours of oestrum all showed only markedly atrophic corpora in the ovaries. The frequency of occurrence of Stage VII and Stage III. ovaries in slaughter house material/

material indicated that these two stages together occupy about 3 days of the cycle so that an interval of this length separates the commencement of regression of the corpus luteum and the following ovulation. Oestrum lasts, on the average, 36 hours and there is ample evidence to show that ovulation takes place before the end of heat.

The shape of the corpus luteum during the early stages of its development is determined largely by the presence or absence of other ovarian structures in proximity to it. When there are no follicles or other corpora near, the corpus is usually more or less spherical, apart from the protrusion from the surface, throughout its development, but when there are adjacent structures they commonly cause distortion of the developing corpus luteum. The fully developed corpus is usually spherical in form.

Vascularisation of the corpus takes place very soon. The wall of the collapsed follicle is creamy-white in colour: as the wall begins to thicken it changes in colour to a deep pink and finally to flesh-red. Extravasation of blood in the protruding part of the corpus gives to this part a colour much deeper than that of the corpus generally. The corpus is fully vascularised by the end of the fourth day of the cycle. Although the term "corpus luteum" is used for convenience there is no suggestion of yellow colour in the fully developed corpus luteum of/

of the sheep. When regression sets in the corpus rapidly loses its deep red colour, becoming cream coloured or faintly yellow: as involution proceeds the colour gradually deepens and finally turns to orange or brown. The last trace of the corpus remains distinguishable as a brown or chocolate patch for several months and perhaps permanently. When the atrophic corpus lies close to the tunica albuginea, as it most commonly does, it is frequently represented on the surface by a small glossy knob, the nature of which is uncertain.

The corpus luteum of the sheep thus seems to contain only a very small amount of lipochrome pigment: this does not affect the colour of the body when it is fully developed but as involution takes place it seems to become gradually concentrated and thus becomes visible to the naked eye.

The fully developed corpus luteum usually possesses a central cavity filled with fluid resembl-:ing the liquor folliculi. The size and shape of this central cavity vary greatly: it may contain as much as a cubic centimetre of fluid. Hammond (66) found that, in the cow, the redistension of the cavity of the corpus luteum by fluid is a normal stage in the development of the body: later on the fluid is resorbed and the central cavity becomes filled with a plug of connective tissue. In the sheep, however, this/

this resorption does not take place until the involution of the corpus is well advanced. Should the animal be served and the corpus luteum become converted into a corpus luteum of pregnancy the fluid remains although there are indications that gradual absorption takes place. Connective tissue, if it is present, is never visible macroscopically in the sheep's corpus luteum.

In Fig. 16 schematic curves showing the rate of growth (diameter) of the corpus luteum and Graafian follicle are given. The size of the fully developed corpus luteum is remarkably uniform, few being less than 10 or more than 13 millimetres in diameter.

B. The Graafian Follicle.

In many mammals, including the cow, it has been found that the Graafian follicles are subject to only slight changes during the interoestrous period: during or immediately after oestrum, however, there is a very considerable increase in the size of the largest follicles, culminating in rupture. In the sheep, exactly the same sequance of events has been found to occur.

According to Quinlan and Maré, a rapid enlargement of the follicles destined to rupture at the next oestrum takes place shortly after ovulation, these/

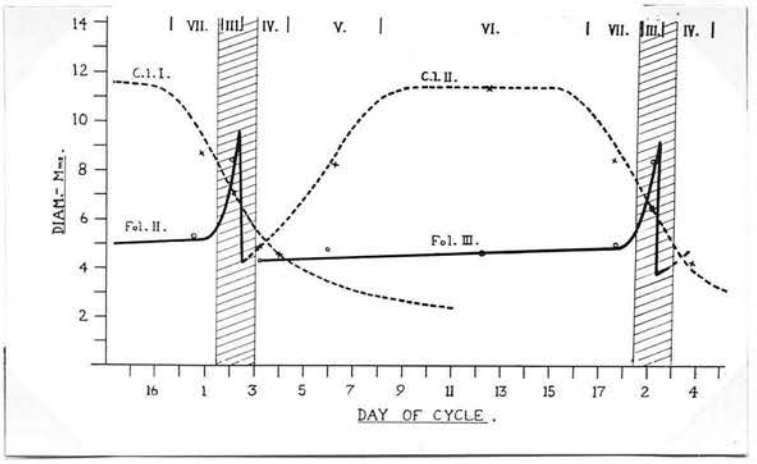


Fig. 16. Growth curves (mean diameter) of ovarian structures (semi-diagrammatic).

××× Mean group values for diameter of corpora lutea.

ooo Mean group values for diameter of largest Graafian follicle.

The cross-hatched areas indicate the oestrous periods.

these follicles continuing to grow, but slowly during the remainder of interoestrum. This has not been confirmed. The measurements that have been taken show that there is a very slow increase in the mean size of the largest Graafian follicle throughout the interoestrum: but this increment seems to be due not so much to an increase in the diameter of the largest follicle in each ovary as to the greater frequency of occurrence of large follicles in the later stages. In every stage of interoestrum ewes are found which show only very small follicles in the ovaries while others contain follicles as much as 7 mm. in diameter. Towards the end of the inter-:oestrum a much greater proportion of the ovaries show large follicles. It thus appears that during interoestrum one or more follicles grow to a certain size, i.e., about 5 to 7 mm.; when follicles of this size are present at the beginning of the inter-:oestrum they do not increase further in size.

The apparent changes in the ovaries must, however, be accepted only with considerable reserve until the histological changes have been examined, for it has been shown (Evans and Swezy (53)) that in the rat, guinea-pig, dog, cat and man, the matura-:tion of a group of follicles involves the destruction of the other smaller follicles in the ovaries, these being replaced by an ovogenetic wave after ovulation.

At about the time when heat begins the

largest/

largest follicles commence to grow rapidly, (Fig. 16), this growth culminating in rupture about 30 hours later. The capsule becomes excessively thin so that the follicle appears to be much darker coloured than before. Traversing the capsule, outlined against the dark background of the liquor folliculi, may be seen a number of small, branching blood vessels. Near the actual point of dehiscence slight extravasa-:tion of blood takes place from these capillaries. Rupture always takes place in the centre of the free side of the follicle: it is preceded by the elevation of a small papilliform area of the follicle above the general surface. The bursting of the follicle thus resembles closely the bursting of a rubber bladder when it is dilated excessively. There can be no doubt that the final stages in dehiscence are purely mechanical and due to the pressure of the fluid within the follicle.

The size of the follicle at the time of rupture seems to vary greatly. The minimum is pro-:bably about 8.5 mm. and the maximum about 15 mm. The most frequent dimension seems to be about 9 to 10 mm. It is probable that the occurrence of particularly large follicles is due to definitely delayed ovulation for in many cases an extremely large follicle has been found to exist in the same ovary as a corpus luteum 2 to 3 days old (see Figs. 11_9 and 12_1). In the sow (Corner (44)) and in the rat (Long/

(Long and Evans (95)) it has been found that all the follicles rupture at the same time.

That these follicles do ultimately rupture and do not become atretic is indicated by the circumstance that they do not occur after about the 3rd day of the cycle. That they do occasionally fail to rupture, however, is indicated by the occurrence of follicular cysts. These may be from 10 to 20 mm. in diameter: each is surrounded by a fibrous capsule sometimes as much as 3 mm. thick. They are of rare occurrence, however, only 6 cases were encountered in the 2,000 odd pairs of ovaries examin-:ed. These cysts do not interfere with normal ovarian changes: corpora lutea in various stages of development may co-exist with them in the same ovary.

The pre-ovulation swelling does not appear to have been observed heretofore in the sheep.

The microscopical changes in the ovary are being studied in detail but these studies are not yet far enough advanced to justify any report upon the findings.

3. THE TIME OF OVULATION.

Quinlan and Maré (130) conclude that ovulation takes place in the Merino ewe near the end of the heat period, i.e., about 40 hours after the commencement/

commencement of heat. This conclusion appears to be based upon the autopsy of 6 ewes killed during oestrum. They believe that a causal relationship exists between heat and the presence of mature follicles and that heat passes off shortly after the rupture of these follicles. The occurrence of exceptionally long heat periods they ascribe to delayed ovulation.

Cole and Miller (41) found that six Rambouillet ewes killed during the first 24 hours of heat had not ovulated, while 8 out of 12 ewes killed during the second day had ovulated. The time of ovulation thus seemed to lie at about 24 to 36 hours. The duration of heat in these ewes was 30 to 40 hours.

Allen et al. (2) found that ovulation did not occur until $23\frac{3}{4}$ hours after the beginning of oestrum.

The most extensive data on this subject are those given by Ivanow (76). His data may conveniently be presented in tabular form.

Table 1.

Time after commencement of heat.	No. of animals.	No. not yet ovulated.	Number ovulated.
1 - 24 hours	43	42	1
25 - 48 "	46	4	42
49 - 72 "	9	1	8
73 - 96 "	1	0	1

From/

From the above table it will be seen that ovulation almost invariably took place 24-48 hours after the commencement of heat although rarely it might be delayed.

Table 2.

Time after end of oestrum.	No. of animals.	No. not yet ovulated.	Number ovulated.
1 - 24 hours	28	2	26
25 - 48 "	30	0	30
49 - 72 "	28	0	28
73 - 96 "	28	0	28

Comparing tables 1 and 2 it may be concluded that heat lasted about 36 hours in these ewes and that ovulation took place during the last few hours of heat.

Out of 185 ewes served (presumably artificially inseminated) during the first six days after the end of heat only eight conceived: six of these were inseminated less than 24 hours after the end of heat. Ivanow concludes from this that the ovum retains the capacity to be fertilised and to develop for only a short time after extrusion. This conclusion is not altogether justified, however, for it makes no allowance for the possible influence of the cervical and other secretions upon the sperm.

Hammond (66) has shown that the time of ovulation in the cow is 24 to 48 hours after the commencement/

commencement of oestrus, i.e., it takes place after the end of heat, which normally lasts only about 17 hours.

The investigations of the present author did not include any direct attempt to determine the time of ovulation. Out of the five ewes killed during oestrum (see Appendix Table 1) only one (killed at 18 hours after the commencement of heat) had ovulated. This ewe showed a just ruptured and only partiallly collapsed follicle in one ovary and in the other ovary a follicle on the point of rupturing. The other four ewes all showed very large follicles in their ovaries and no recent corpora lutea.

Certain considerations suggest indirectly, however, that ovulation may occur earlier in Scottish sheep than appears to be the case in the Merino and in the Russian breeds examined by Ivanow. It will be recollected that all the material examined at the abattoir was classified according to the condition of the ovaries and that, in order to determine what period of the dioestrous cycle was represented by each of the ovarian Classes, it was assumed, on the basis of published observations, that ovulation took place about 30 hours after the commencement of heat. In dealing with the cyclical changes in the other reproductive organs, particularly those in the vagina, it will be shown that the changes in abattoir material/

material appeared to occur somewhat later in the cycle than would be expected on the basis of findings in the experimental ewes. The logical interpretation of this discrepancy is that, in estimating the stage of the cycle represented by slaughter-house ewes, ovulation has been assumed to occur later than is actually the case. The discrepancy is nullified if ovulation is taken to occur about 18 hours after heat begins.

That ovulation may sometimes occur as early as this is indicated by ewe 736 killed 18 hours after the commencement of heat: ovulation had just taken place. On the other hand experimental ewe 826 had not ovulated 24 hours after the commencement of heat, nor was ovulation imminent. It has already been indicated that an interval of as much as two days may separate the times of rupture of two follicles of the same crop.

There is no positive evidence to support Quinlan and Maré's contention that heat passes off soon after rupture of the follicle and that long heat periods are due to delayed rupture. While it is true that prolonged heat in the rabbit, ferret, etc., is associated with the persistence of unruptured follicles, and nymphomania in the cow with follicles that have become cystic, there is no reason to believe that heat in the ewe may not continue long after/

after rupture has taken place. In many mammals heat does not commence until after the follicle has ruptured.

It is the opinion of the writer that heat ends when the concentration of the heat-causing hormone falls below a certain threshold value, and consequently that the duration of heat is determined by the amount of this hormone produced during the maturation of the follicle: the time of rupture, when this lies within normal limits is not thought to influence the duration of heat to any extent. Brambell and Parkes (33) have shown that in the mouse the heat stimulus is initiated two days before oestrum actually commences and that ovariectomy after this time does not prevent the onset of oestrum.

If Quinlan and Maré are correct in their conclusion that prolonged heat is due to delayed rupture, then the life of the corpus luteum produced under such circumstances must be curtailed for it has been shown that the duration of heat is not correlated with the duration of the cycle and the onset of a new heat period does not take place until the corpus luteum of the previous cycle has degenerat-
:ed.

Ovulation in the sheep seems to be entirely spontaneous and independent of coitus. Marshall (101) concluded that late in the breeding season the extra stimulus of coitus may be required before ovulation can/

can take place. This conclusion appears to have been based largely upon the finding of unruptured follicles in the ovaries of ewes killed not more than 24 hours after the commencement of heat: it is not certain that ovulation normally takes place before this time. It has been shown that in the slaughter house material ovulation continued to take place in all mature ewes until mid-February and in some until April. The ewes upon which Marshall based his conclusions were killed in February, a time which he thought to lie outside the normal breeding season.

Marshall was also of the opinion that although ovulation occurred spontaneously during the normal breeding season, its occurrence could be hastened by coitus. Ivanow (76) has shown that coitus has no effect whatever upon the occurrence of ovulation. It has been shown (page 115) that sterile coitus does not affect the duration or periodicity of heat.

VI : CYCLICAL CHANGES IN THE UTERUS.

1. THE STRUCTURE OF THE UTERUS.

The uterus of the sheep is of the bicornuate type, but the lumina of the two horns are confluent for only a very short part of the uterus in which the cotyledons are only slightly developed. The "body" of the uterus is thus not actually part of the functional organ. The two horns are joined to one another posteriorly by a common muscular coat for a little more than half their length.

The mucosa is thrown up into a great number of prominences about 5 mm. in diameter and 3 mm. high: these "cotyledons" as they are called, are the areas to which the foetal membranes become attached (see Figs. 24 and 52 on pages 185 and 296). There are about 60 cotyledons in each horn and in the virgin animal they are arranged in four rows, continuous with the four main folds of the Fallopian tube mucosa.

The mucosa may be divided roughly into two layers, a thin superficial layer of dense stroma and a deep, thick layer of much looser texture in which there are a great many small lymph spaces. This layer may also contain intercellular deposits of hyaline material staining with eosin and similar stains. The chemical nature of this substance has not/

not been determined. The cotyledons are essentially thickenings of the superficial dense stroma layer: in mature animals, however, the deep, loose layer is also considerably thickened and the muscular layer may send trabeculae into the cotyledon, usually along the course of the blood vessels.

The openings of the uterine glands are confined to the intercotyledonary areas. The glands are of the branched tubular type, each gland breaking up into a great number of branches in the deeper stroma. The branches are of less diameter than the main duct and there are no terminal acini. As a rule the main duct commences to branch some consider-:able distance below the surface so that the branches are confined to the loose stroma and are mostly adjacent to the muscular coat. As already stated, there are no glands in the cotyledonary areas: frequently, however, the branches of adjacent glands extend into the loose stroma at the base of the cotyledon.

In ewes which have been pregnant at some time there is commonly a layer of relatively loose stroma between the dense layer and the epithelium.

Melanoblasts are frequently present in the stroma, particularly in the cotyledonary areas. They are usually concentrated immediately beneath the epithelium but in heavily pigmented uteri they extend deep/

deep into the stroma, along the course of the blood capillaries: they are never found in the muscular coat and rarely in the loose stroma layer. (See also page 188) Accumulations of amorphous brown pigment are commonly found, especially during the anoestrum following pregnancy. This pigment is derived from extravasated blood. In a number of ewes killed during the anoestrum following pregnancy in the preceding winter, peculiar pigmented cells, round or oval in form, without dendrictic branches and of a uniform, non-granular, yellow-brown colour, were found. These appear to be analogous to the pigment cells found in the uterus of the mouse and other animals after pregnancy which have been describ-:ed by Benazzi (17, 18).

The capillaries in the cotyledon are only slightly branched, and are mostly radial (i.e., perpendicular to the epithelium) in arrangement. They come close to the epithelium and double back sharply.

There are two chief muscular coats, an internal coat, in which the fibres are nearly all arranged in a circular manner, and an external in which the fibres are nearly all longitudinal. These layers are separated by the vascular layer. The inner coat is continuous round each horn: the outer coat on the other hand merges on each side into the broad ligament and does not extend between the cornua where/

where these are joined to one another, in fact it is mainly the continuity of this coat from one horn to the other that binds the two together. Thus, except near the tip of each horn, the longitudinal coat is confined to the ventral and lateral parts of the uterus. Near the tip of the horn the longitudi-:nal coat extends most of the way round but it is never complete. The regularity of the coat is lost near the broad ligament, quantities of connective tissue intervening between the muscle fibres, which become irregular in arrangement.

The vascular layer is traversed by a great many muscle fibres, nearly all lying in the transverse plane but otherwise arranged in all directions. They thus form a network round the blood vessels.

In virgin ewes the blood vessels of the vascular layer are relatively inconspicuous but in a ewe which has borne lambs these vessels are heavily fibrosed. The uterus of such a ewe may be disting-:uished readily by the white, cord-like blood vessels which may easily be seen and felt through the external muscular coat.

The uterus is lined throughout by a simple columnar epithelium. The nuclei in the cells lie at all levels except near the free end. It was probably this fact that led Marshall (101) to believe that there were two rows of cells. The epithelium of the/

the glands is also of columnar or cubical type: there can be no doubt that the glandular epithelium is identical in form and function with that of the general surface.

Cilia have not been found in the sheep's uterus, either in the mature animal or during pre-pubertal or foetal life.

Between the epithelial cells, in the stroma immediately beneath the epithelium and in the uterine lumen, are found peculiar, minute, rod-like bodies staining intensely with Heidenhain's haematoxylin but not with other stains. Bonnet (30) found these bodies in the "uterine milk" produced during pregnancy and named them "stäbchen". Smith (155) states that they are probably crystals of calcium oxalate but does not give any evidence in support of this contention. While they are of crystalline form, they cut readily and there was never any evidence of tearing in sections that contained them, such as occurs when crystals of fixing agents are left in the tissues. They were not dissolved out by any of the fixatives used. Their presence bears no relation to the phases of the oestrous cycle and they are some-:times present in the uteri of prepubertal animals.

In the new born ewe lamb the cotyledons are already formed but are not differentiated structurally from the rest of the mucosa. The entire mucosa is composed of very loose connective tissue, there being no/

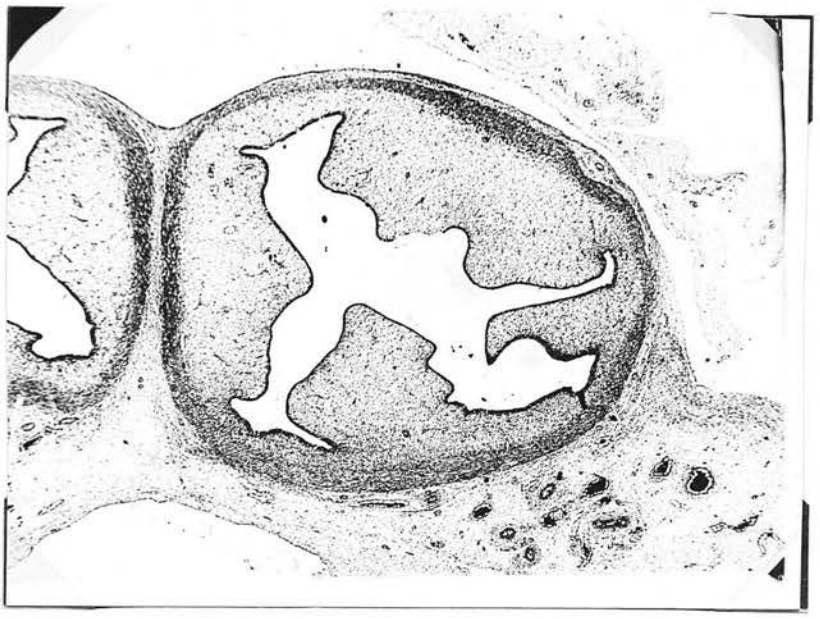


Fig. 17. Section through uterus of a new born lamb, showing absence of differentiation in the mucosa and absence of glands. Note that the pigment cells are distributed throughout the mucosa.

no differentiation of dense and loose zones and no glands are present. The external longitudinal muscle coat is also almost entirely absent. (Fig. 17)

2. STRUCTURAL MODIFICATIONS OF THE UTERINE MUCOSA.

Marshall (101) has given a brief account of the changes in the uterus of the ewe during the oestrous cycle. When the present investigation was commenced Marshall's paper constituted the only published account of these changes, apart from the observations of Bonnet and others referring mainly to the uterine pigment. Recently, Casida and McKenzie (37) have published more detailed observations on this subject but the number of animals autopsied was only nine: all these ewes were of uncertain history. Casida and McKenzie's account refers mainly to uterine structures not discussed by Marshall, but where the two accounts overlap they are mutually contradictory.

Except in a very few points the present author is unable to agree with either of the accounts mentioned. It is believed that these discrepancies may be ascribed to the remarkable degree of both regional and individual variation which has been found to exist.

A./

A. Gross Changes.

During the breeding season the uterus is slightly larger and heavier than in anoestrum. This has been shown to be due chiefly to slight oedema during the breeding phase but there is also slight growth of the mucosa. The anoestrous uterus is also rather anaemic in comparison with that of the breed-:ing ewe: the difference is a slight one, however.

During oestrum and early metoestrum the large blood vessels of the uterus are somewhat con-:gested. The uterine mucosa, also, may appear flushed, particularly on the cotyledons. This flushed condition of the cotyledons is found at other times, however, and has been shown to be due to the collection of extravasated blood beneath the epithelium: thus it is not a true indication of the state of the capillaries. It has been shown by histological sections and by uterine smears that slight haemorrhage takes place occasionally from the uterine mucosa during oestrum or early metoestrum, but in only two instances out of the total of about 2,000 uteri examined was sufficient blood present to be detected with the naked eye. About 120 of the ewes were killed during oestrum and early metoestrum.

There is never any accumulation of fluid in the uterine lumen during oestrum such as is found in the rat and mouse.

It was found that the cervical and vaginal muscles relax during oestrum, but there was no evidence of a similar relaxation in the uterine muscle. The spontaneous activity of the uterine muscle seemed to be least during oestrum but this conclusion is based only on casual observations of the whole uteri when removed from the body: no controlled observations on the subject have been made.

Marshall (101) found that the amount of uterine pigment present differed according to the stage of reproductive activity. This has not been confirmed (see page 194).

B. Microscopical Changes.

i) Changes in vascularity.

During oestrum there is a considerable increase in the size and blood content of the uterine capillaries: this increase is especially marked in the mucosa of the cotyledons, but is present also in the intercotyledonary mucosa and in the capillaries of the uterine muscle. Congestion usually commences about 12 to 24 hours after the beginning of heat, i.e., distinctly later than in the vagina. The congestion persists for about 24 hours and then disappears gradually: the normal condition is reached about the third day of the cycle, i.e., some time after the end/

end of heat.

When congestion is at its height, a few of the cotyledonary capillaries may break down where they double back immediately beneath the epithelium. Less frequently, extravasation of blood may take place deeper in the mucosa. The extravasated blood collects beneath the epithelium and, if the latter is denuded, as is commonly the case in oestrum, some of the blood escapes into the uterine lumen.

It would appear from the observations of Marshall that extravasation of blood almost invar-:iably takes place. This is quite definitely not the case: breakdown of the capillaries is the exception rather than the rule, and it rarely takes place in more than five or six of the cotyledons.

It is to be inferred also, from Marshall's account, that congestion of the uterine mucosa is a pro-oestrous phenomenon as is also the period of capillary breakdown, although the latter may be delayed until oestrum has commenced. This also has not been confirmed. Congestion rarely commences before the beginning of heat and extravasation of blood is certainly a late oestrous or early met-:oestrous phenomenon. Casida and McKenzie (37) state that vascular congestion is a metoestrous phenomenon.

During anoestrum the capillaries of the uterine/

uterine mucosa become reduced both in size and in number. This is conspicuously the case when an-:oestrum follows pregnancy for the whole mucosa becomes markedly atrophic in the later stages of involution.

ii) Oedema.

The dense stroma does not contain lymph spaces of any size or in any number, but the loose connective tissue of the deep stroma is usually fairly oedematous. Oedema is least during anoestrum, particularly where this follows pregnancy. During the whole of the dioestrous cycle there is a marked increase in the size of the lymph spaces but this is especially noticeable during oestrum and early met-:oestrum, i.e., during much the same period as that during which the uterus is congested. The dense stroma is only slightly affected.

iii) The surface epithelium and the epithelium of the uterine glands.

The epithelium of the uterus of an immature or anoestrous ewe is composed of cubical or low columnar cells of very uniform type. The actual shape of the cell and that of its nucleus are deter-:mined by the physical stresses to which the cell is subjected, i.e., where the epithelium is folded the cells are of narrow columnar form with elongated nuclei, but where the epithelium is stretched, as

where/

where it covers a cotyledon, the cells are cubical and the nuclei round. In extreme cases the cells are attenuated laterally, and become fusiform. Nearly the whole of the cell body is taken up by the nucleus. What little cytoplasm there is, stains deeply and is of uniform consistency, i.e., it is not granular or vacuolated. There is no suggestion of secretory activity in the cells. With the approach of the breeding season the height of the epithelium increases without much change in the character of the cells.

The typical epithelium of dioestrum is composed of tall columnar cells (see Figs. 18 and 20) twice or three times the height of the same cells during anoestrum. The nucleus is larger than in anoestrum, is elongated, and occupies a position about half way up the cell. The cytoplasm is usually rather coarsely granular or vacuolated and faintly eosinophilic. There can be no doubt that these cells are of secretory nature. The nature of the secretion is uncertain, however: it is pre-:cipitated by corrosive sublimate but only poorly by picric acid, and it is largely dissolved out by absolute alcohol when this is used as a fixative. At the free end, the cell wall is composed of some substance which stains intensely with iron haematoxylin. Between the cells this material is thickened,/

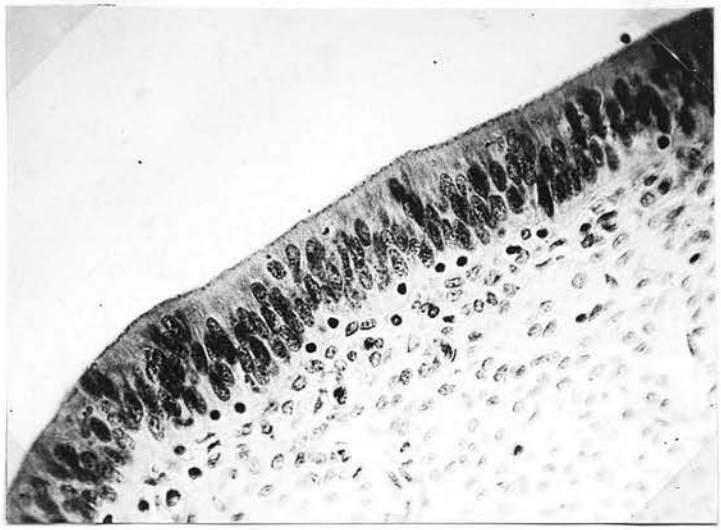


Fig. 18. Epithelium covering a cotyledon. Ewe killed in early metoestrum. x 350. (Figs. 18, 19, 22 and 23 were taken under identical magnification.)

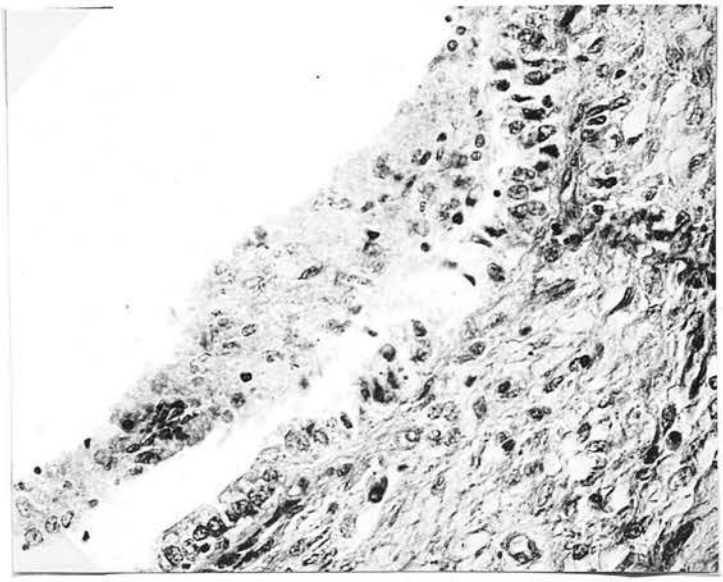


Fig. 19. Showing degeneration of the epi-:thelium covering a cotyledon in a ewe killed in oestrum. x 350.

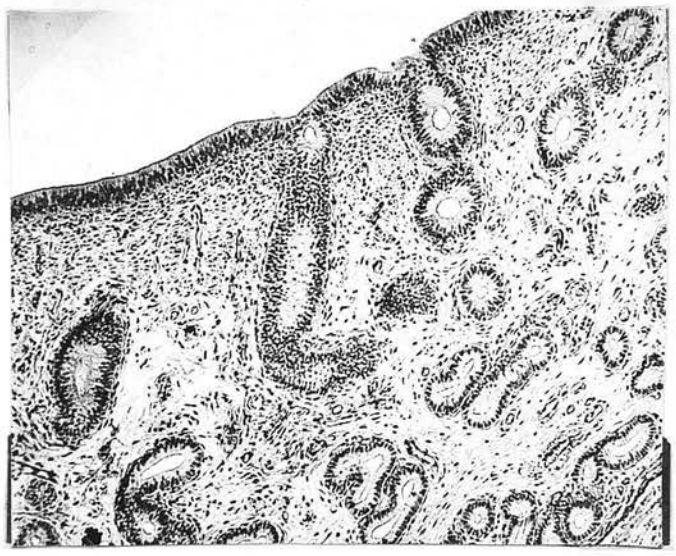


Fig. 20. Uterine mucosa of a ewe killed in dioestrum, showing high intact epithelium of surface and glands.

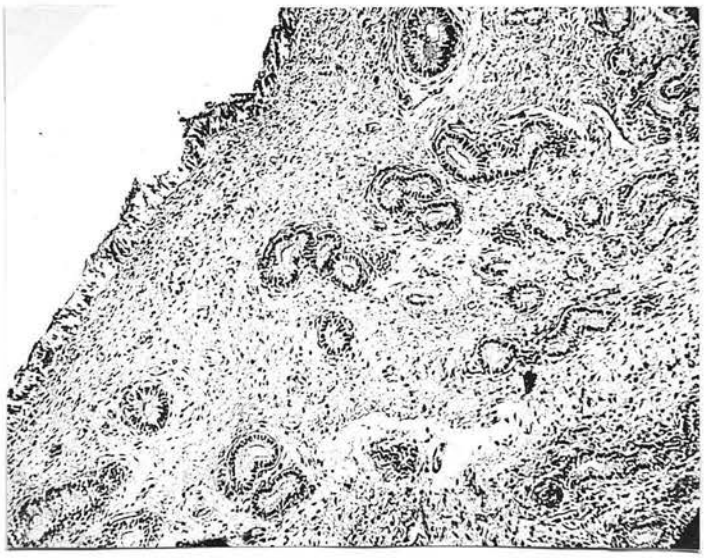


Fig. 21. Uterine mucosa of a ewe killed during heat showing degeneration of surface epithelium and shrunken glands. (Same magnification as Fig. 20.)

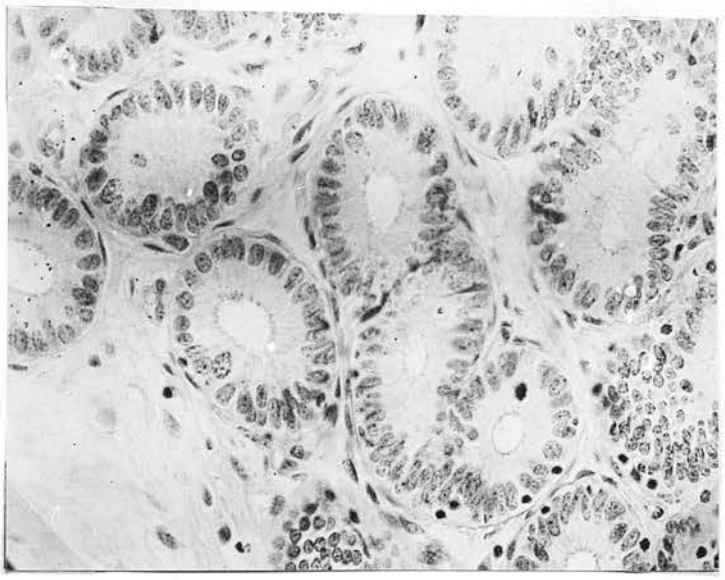


Fig. 22. Uterine glands of a ewe killed in early metoestrum: note mitotic figures. x 350.

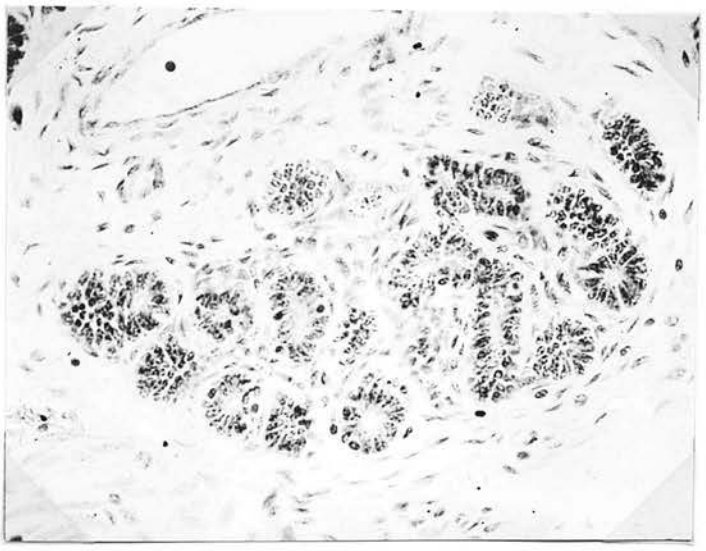


Fig. 23. Uterine glands of a ewe killed in anoestrum. x 350.

thickened, and gives a beaded appearance to the free edge of the epithelium.

The epithelium rests upon a well defined membrana propria.

The epithelium of the uterine glands is similarly constituted: near the mouths of the glands it is higher and more regular than on the surface but in the deep tubuli the cells are usually more cubical in form. During the dioestrous cycle the uterine glands show a well defined lumen through-:out. In anoestrum the epithelium is reduced to a very low level and the lumen, except in the main duct, is almost completely occluded (C.f. Figs. 22 and 23).

There is always a small amount of secretion both from the surface epithelium and from the glands. In addition there is constant extrusion of nuclei from the cells and presumably such cells are them-:selves extruded afterwards. But while these processes occur at all times they are essentially typical of oestrum. During this phase there is intense secretory activity in the epithelia of the surface and of the glands. Secretion is followed by a phase of degeneration. The cells become greatly reduced in height and become irregular in arrangement. Considerable tracts of the surface epithelium may be completely denuded, and, where this occurs, there is usually/

usually some denudation of the underlying stroma. The basement membrane is very largely destroyed. (C.f. Figs. 18 and 19; 20 and 21.) The degenerative changes are always strictly localised, however, and it is common to find areas of the uterus of a ewe killed in oestrum covered by the fully developed epithelium characteristic of dioestrum. In the glands the degenerative changes are not so marked. Individual cells are cast off and nuclear extrusion is very common but there is very rarely any consid-:erable denudation of the epithelium.

Oestrum is thus the secretory phase in the uterus, and it appears to be quite just to include the cast off epithelial cells and the products of cell degeneration as parts of the secretion.

The secretory and degenerative changes in the uterine epithelium somewhat precede the degenera-:tive changes which occur in the vaginal epithelium: they coincide roughly with the secretory phase of the cervical mucosa. This coincidence might have been anticipated for the uterine epithelium differs little in morphology from that of the cervix and the two are derived from the same embryonic source, the epithelium of the Müllerian duct. The chief differ-:ence is in the nature of the secretion, for while the cervix produces mucin, this is never found in the uterus. The chemical nature of the uterine secretion has not yet been elucidated, but the fluid is no doubt/

no doubt similar to the more copious uterine secre-:tion that is produced during pregnancy ("uterine milk").

It is impossible to define exactly the limits of the uterine degenerative phase for these seem to be subject to a great deal of variation. It seldom commences before heat begins and its commence-:ment is rarely delayed after the time of ovulation. The phase seems to be of very short duration for restorative changes are usually noticeable by the third day of the cycle, i.e., about 24 hours after the end of heat, and the maximum degree of develop-:ment of the epithelium is reached by about the sixth day of the cycle. Where extensive destruction of the surface epithelium takes place, however, the process of repair is not completed, apparently, for some time, and thus areas of the uterus, particularly on the cotyledons, are found during dioestrum covered by a very rudimentary epithelium.

Marshall did not observe any definite changes in the uterine epithelium, but thought that there was some degeneration in oestrum. The glands, he believed, showed an increase in size during pro-oestrum and oestrum. Casida and McKenzie likewise failed to observe any definite cyclical changes in the structure of the uterine epithelia, but thought that the glands showed increased coiling and branching/

branching during dioestrum. The writer has not confirmed the last finding.

iv) The stroma cells.

While there are quite considerable changes in the intercellular spaces of the stroma during the dioestrous cycle there are few definite changes in the actual cells. The nuclei are larger and more vesicular during dioestrum than during oestrum but there is no appreciable increase in the size of the cells or in the nature of their cytoplasm.

v) Mitotic activity.

The degree of mitotic activity in the epithelia of the uterus and of the uterine glands is very definitely related to the phases of the reproductive activity. Cell division is never very intense; the greatest number of mitotic figures observed in the surface epithelium was about 15 to 20 per centimetre of section 6 μ thick. Most activity is found in the gland mouths. Mitosis is more common in the intercotyledonary zones than on the cotyledons and is comparatively rare in the deep gland tubuli.

During the true anoestrum there is practically no cell division: the few mitotic figures that were seen during this phase were in the surface epithelium.

Oestrum, it has been noted, is associated with considerable secretory activity and breakdown in the/

the epithelia of both the uterine cavity and the glands. During this phase mitosis is almost entire-:ly in abeyance although a few divisions occur in the epithelium of the gland mouths. At about the time of ovulation mitotic activity increases greatly and it remains at a high level until about the fifth day of the cycle. Thereafter mitotic figures be-:come rarer and a state of almost complete quiescence is reached by about the ninth day of the cycle. Casida and McKenzie found mitotic figures during the same period and not at other times. The fully developed corpus luteum spurium is thus associated with a highly developed uterine and glandular epithelium in which mitotic activity is at a very low level. The cessation of mitosis is not occasioned simply by the high degree of development of the epithelium however, for full hypertrophy of the epithelium is commonly reached by about the fourth day of the cycle when mitotic activity is at its height. The nucleus rises in the cell body before division.

Mitotic division of the nuclei of the dense stroma of the cotyledons is a rare phenomenon during the oestrous cycle and in the loose stroma it is even rarer. The greatest amount of division occurs during the same period in which mitosis occurs in the epithelia. Marshall (101) concluded that/

that there was a wave of nuclear division in the stroma of the cotyledon during pro-oestrum and early oestrum, that is, during the phase when development of the blood vessels was said to take place. He did not observe mitotic figures, however, and concluded that nuclear division took place amitotically. The present writer has been unable to find any evidence of considerable nuclear division in the stroma dur-:ing any phase of the cycle and there is nothing to indicate that division may be amitotic. Moreover, since there is no evidence of nuclear disintegration in the stroma, any considerable cell division would lead to progressive increase in the size of the cotyledons during the breeding season and this does not occur.

vi) Lymphocytes and leucocytes.

During the puerperium there are frequently heavy infiltrations of polymorphonuclear neutrophilic and eosinophilic granular leucocytes into the uterine mucosa. These may be found in the mucosa for several months after parturition. During the di-:oestrous cycle, however, no leucocytes at all were found in the mucosa. Lymphocytes are almost invar-:iably present, however. They are distributed throughout the mucosa, showing only a slight tendency to concentrate beneath the epithelium. They invade the epithelia of both the general surface and the glands/

glands, and pass through into the lumen of the uterus. There was no evidence of any cyclical variation in their number.

VII : CYCLICAL CHANGES IN THE FALLOPIAN TUBES.

The mucosa of the oviducts is covered throughout by very tall columnar ciliated cells. This epithelium is not subject to any striking cyclical alterations during the dioestrous cycle, but there may be changes in cytological detail. The number of oviducts examined so far is small, however, and the fixatives employed for these were not adequate to preserve the finer cytoplasmic structure. It is, therefore, not as yet possible to make any general statement with regard to the oviduct. According to Casida and McKenzie (37) there is an increase in the height of the epithelium during metoestrum but this has not been confirmed.

The Fallopian tubes become congested during the same phase of the cycle in which the uterus becomes congested, i.e., in oestrum.

VIII : THE PIGMENTATION OF THE UTERUS AND FALLOPIAN TUBES.

It has already been noted that the uterus of the sheep is frequently characterised by an intense pigmentation of the mucous membrane. This pigment is perhaps peculiar to the sheep, for, although references to uterine pigment in other mammals are frequent in the literature, in no case does the pigment appear to be of comparable nature. The present writer did not find pigment in the uteri of three goats which were examined but Zietschmann (169) figures the uterus of a goat which was pig-:mented in a manner apparently identical with that in the ewe.

That the different breeds of sheep may vary in respect of the occurrence of this pigment is suggested by the fact that Bonnet (27, 29) found the pigment in only 7% of 200 uteri examined (Merinos and "Zackelschafen"), while the present author has found, from observations on genitalia from some 2,000 Scottish sheep, that more or less intense pig-:mentation occurs in more than 80% of cases. On the other hand, significant differences between the var-:ious breeds examined were not established and pigment certainly occurs in all the common Scottish breeds. The other authors who have described this pigmentation/

pigmentation of the uterus (Kazzander (78) and Marshall (101)) do not, unfortunately, give figures for the frequency of its occurrence.

In what may be described as typical instan-:ces, the pigment is confined almost entirely to the cotyledons of the uterus and to the juxta-uterine parts of the Fallopian tubes (Fig. 25). There may be flecks of pigment in the intercotyledonary areas but these are usually unpigmented. There does not seem to be any tendency for any one part of the uterus to be more heavily pigmented than the others. According to Bonnet the pigment is more intense on that side on which ovulation last took place, or in the pregnant horn, in cases of early pregnancy. There is also, according to this author, a tendency for the horns, rather than the body of the uterus, to be pigmented. These statements have not been con-:firmed and it appears probable that Bonnet drew his conclusions from insufficient material.

For the purpose of tabulation and comparison with other data, the material collected at the slaughter houses was classified with regard to uterine pigmentation into four groups as follows:-

Group 0. The uterus was completely unpigmented or only a few flecks of pigment were present.

" 1. The cotyledons were flecked with pigment and also, perhaps, some intercotyledonary pigment might be present. (Fig. 24.)

Group 2. The cotyledons were deeply pigmented and there might be flecks of pigment between the cotyledons. This group corresponds to the typical case described above. (Fig. 25.)

" 3. Uterus intensely pigmented. Not only were the cotyledons pigmented but there was also considerable pigmentation of the inter-:cotyledonary areas. The entire uterus might be jet black with the exception that the gland mouths, being unpigmented, gave the intercotyledonary pigmented areas a granular appearance.

The percentages of the uteri falling into these four groups, calculated on the entire number of non-pregnant genitalia examined, were as follows:-

Group 0:	19 %
" 1:	13 %
" 2:	50 %
" 3:	18 %

Generally speaking, the Fallopian tubes showed a progressively greater degree of pigmentation in these groups. A few of the Group 0. cases showed slight pigmentation of the oviducts. In the Group 3. cases all the oviducts were correspondingly intensely pigmented. The juxta-ovarian half of the tube was always/

Fig. 24 : "Grade I" pigmented uterus.

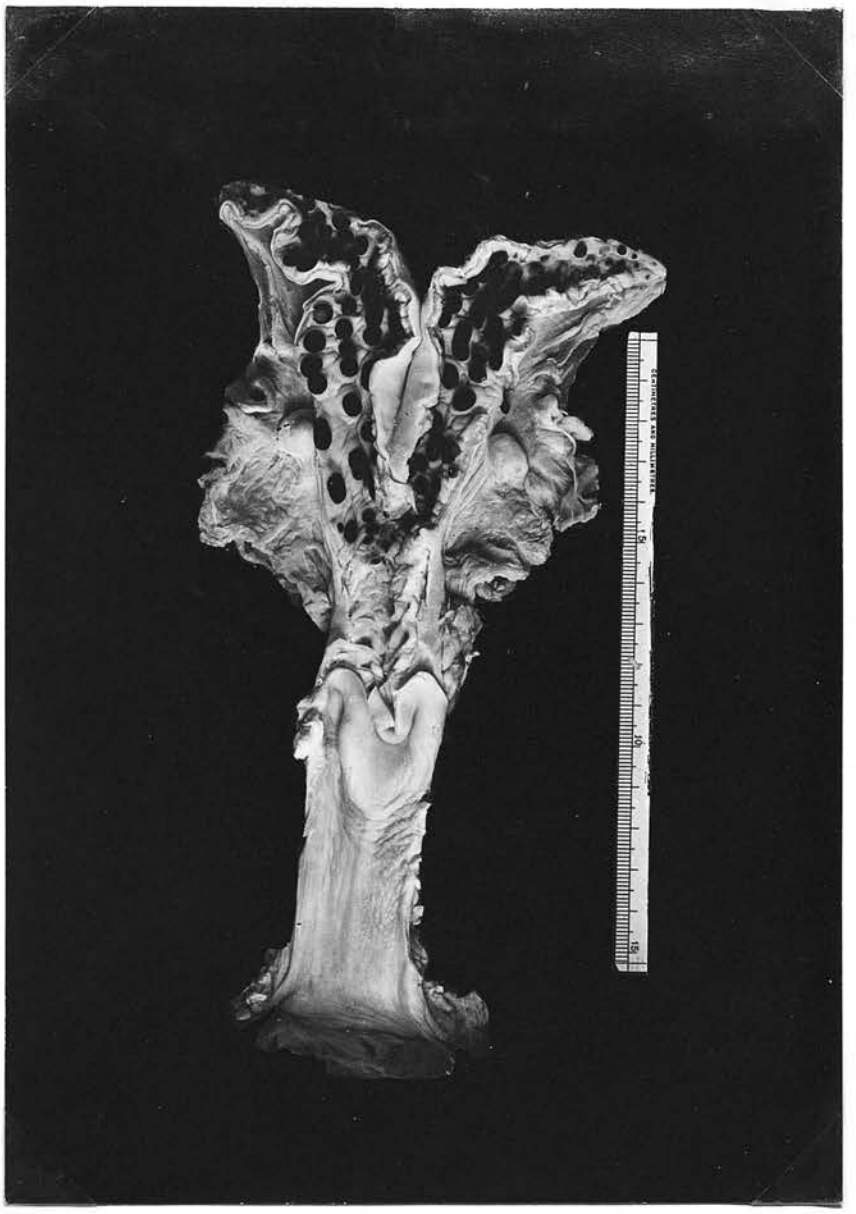


Fig. 25. "Grade II" pigmented uterus. Pigment confined to cotyledons which are uniformly pigmented. This is the type of pigmentation most commonly encountered.

Note also the great length of the Cervical Canal and the strong, muscular folds of its wall, the most external of which constitutes the os externum of the cervix.

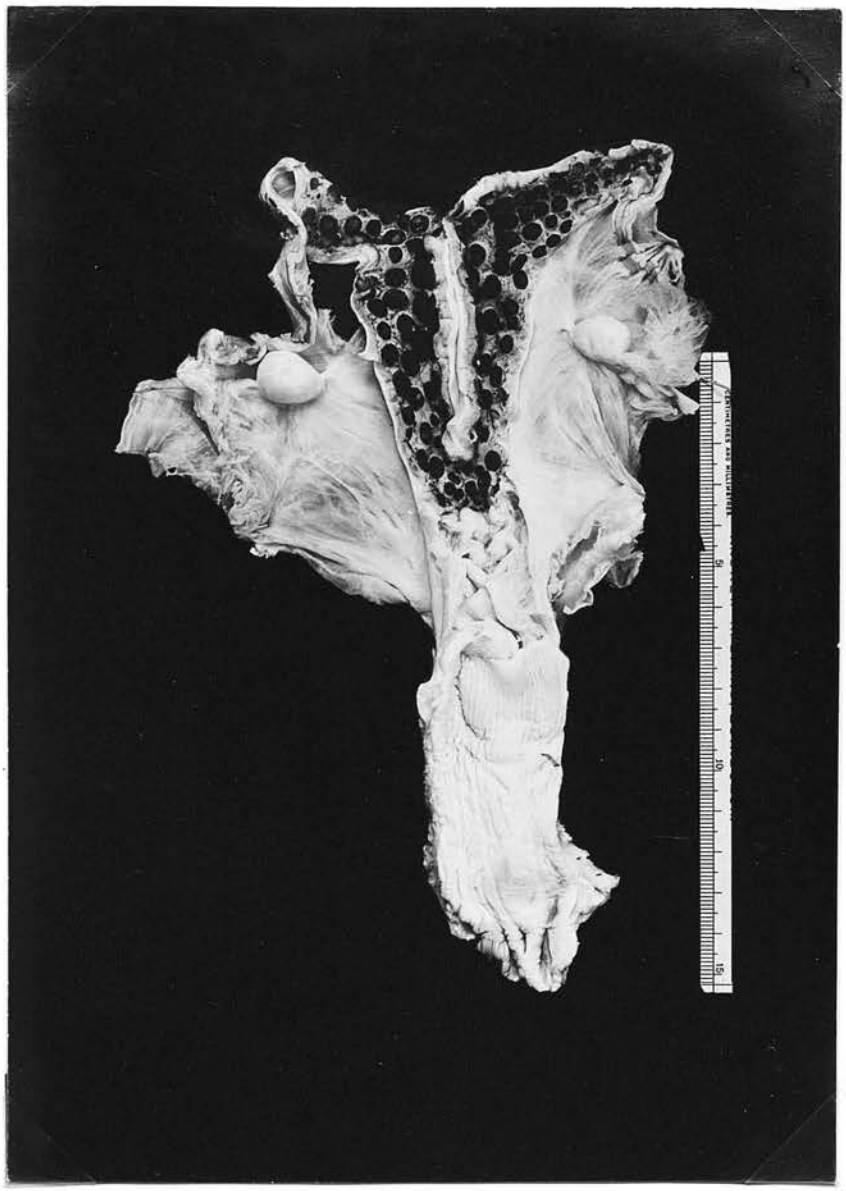


Fig. 26. "Grade III" pigmented uterus: the pigment extends to the intercotyledonary areas.

always less deeply pigmented than the juxta-uterine half and the extreme ovarian end and ostium abdominale were rarely pigmented.

The original description given by Bonnet (27) of the appearance of pigmented areas in histological preparations has been found to be, in all important respects, correct. Bonnet described the pigment of the uterus only, although he recognised that the Fallopian tubes were pigmented. The author has been unable to find any reference to the histological appearance of the Fallopian tube pigment: subsequent workers do not appear to have observed pigment in the oviduct.

Sections through a pigmented cotyledon show that the pigmentation is due entirely to a layer of dendritic pigmented cells (Figs. 27 and 28). These cells are immediately sub-epithelial in position and occupy the interstices of the sub-epithelial layer of connective tissue. Occasional cells are found also in the deeper parts of the stroma. The pigment of the intercotyledonary glandular areas of the mucosa when pigment is present in these areas, is entirely similar in nature.

The structure of the pigmented cell is illustrated in Fig. 29. The form of these cells is best seen in unstained preparations of pieces of epithelium stripped from pigmented uteri and cleared in/

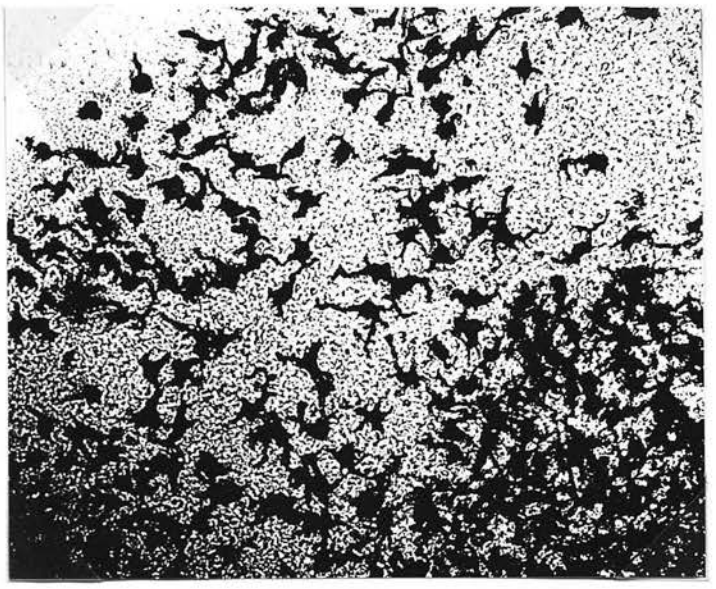


Fig. 27. Thick horizontal section of cotyledonary mucosa showing melanoblasts. Unstained preparation. x 150.

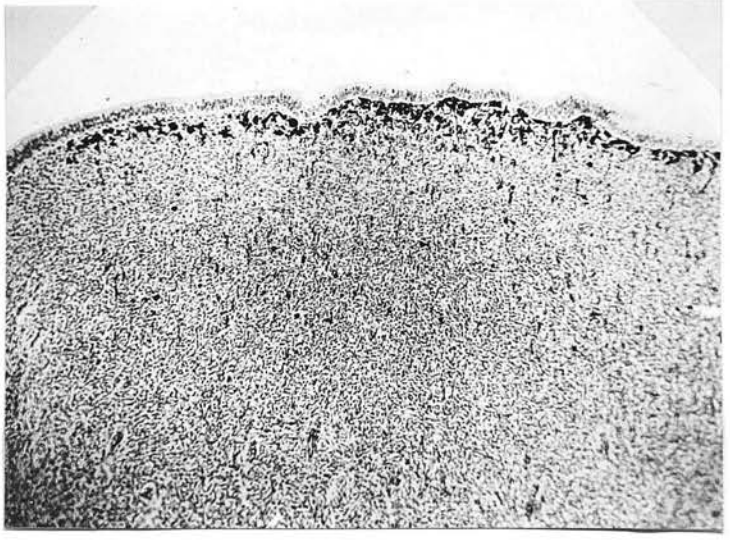


Fig. 28. Vertical section through a pigmented cotyledon.

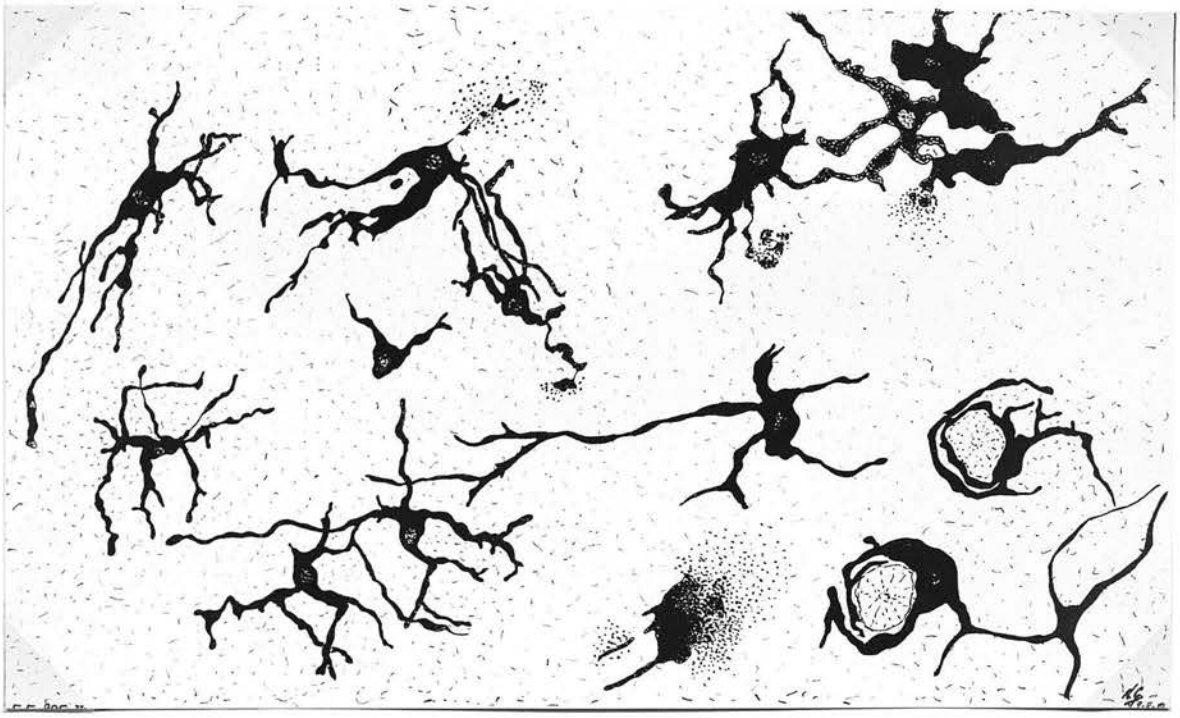


Fig. 29. Drawings to illustrate the morphology of the melanoblasts of the uterine mucosa. Note anastomosis and the tendency to coil round the gland tubuli.

in methyl salicylate.

The cells are rather large in size, the body of the cell measuring, usually, from 12 to 30 μ, the majority being about 18 μ. The great variation in size is associated with, or due to, differences in pigment content, the larger cells being much more heavily pigmented than the smaller.

Each cell gives off a number (1 to 6) of long processes. These may branch repeatedly: the branching is not always simply dichotomous but three or four branches may be given off at once, or on the other hand, side twigs may be produced. The processes are grossly contorted and constricted. Their free ends are usually blunt and dilated like the pseudopodia of an amoeba. This fact, taken in con-: junction with the general conformation of the cell suggests that they are capable of active movement. Also, the fact that the pigment in the Fallopian tubes is usually restricted to the parts adjacent to the uterus suggests that the pigment cells migrate into the oviduct from the uterus. Koller (82) has described mesodermal pigment cells in the fowl and has shown that they are actively motile in tissue-culture. These cells appear to be identical with those which are found in the sheep's uterus. Motility has not been observed in material examined fresh in warm Locke solution, however, nor has streaming of the pigment been seen, and it may be that the/

the cells are stationary, the processes picking their way between stroma cells during their growth and thus acquiring their constricted and contorted appearance. The length of the processes, especially those which descend into the deeper stroma, is sometimes very great. Occasionally, processes are found extending throughout the depth of the stroma of the cotyledon, to the loose stroma layer, a length of some 4 mm. The actual cells were rarely found more than a millimetre below the surface. The horizontal processes are usually much shorter than the vertical but are nevertheless 3 to 4 times the length of the cell body.

In addition to the intra-cellular pigment Bonnet (27, 29) described extra-cellular, sharp-contoured, glistening, pigmented bodies occurring in the deeper parts of the stroma. He concluded that the pigment was of haematogenous origin and was derived from the haemoglobin of extravasated erythro-:cytes, although he had not, at that time, observed extravasated blood in the tissues.

These masses of pigmented material, he thought, were taken up by wandering phagocytic lymphoid cells occurring in the uterine stroma and carried by these to the epithelium. The latter formed a barrier to further migration and the cells flattened out against the epithelium and anastomosed with one another. In the cotyledonary areas migration took place principally along the blood vessels but in the intercotyledonary/

intercotyledonary areas, mainly along the uterine glands. In the oviducts migration seemed to take place along the length of the tube and not vertically to its mucosal surface. The "lymphoid cells" con-:cerned in the phagocytosis of the pigmented material he thought to correspond to the eosinophiles of Ehrlich. Bonnet believed that Ehrlich's eosino-:philic cells were modified forms of the ordinary connective tissue cells (fibroblasts.).

Kazzander (78) observed extravasated blood in the uterus of the sheep, and described intermed-:iate appearances in the transformation of blood into pigment. He concluded that each erythrocyte retained its identity when transformed into pigment. The pigment was thought to be extra-cellular, the appearance of cells being due, according to this author, to the grouping of pigment granules round stroma nuclei. Kazzander's descriptions do not agree with the more detailed observations of Bonnet and subsequent workers have confirmed the descriptions of the latter author. There is little doubt that Kazzander's observations were faulty. Macroscopic appearances similar to those described by Kazzander do occur but the pigment in these cases is almost certainly of a different nature. Marshall (101) confirmed Bonnet's observations.

The close similarity between the uterine pigment cells and the melanoblasts of the skin, lamina/

lamina fusca of the eye, etc., together with the absence of any suggestion of intermediate stages between erythrocytes and pigment cells, led the writer to investigate more carefully the exact nature of this pigment and its relation of reproductive activity.

It has been found that the pigment is by no means confined to the times of heat and early pregnancy as was supposed by previous investigators, but occurs at all times without relation to the sexual state of the animal. Neither the presence nor the amount of pigment bears any relation whatever to the stages of the dioestrous cycle, or to anoestrum. Pigment occurs not only in the prepubertal animal but also in the foetal lamb. In animals that have not become pregnant the distribution is nearly always according to the "type" described above, i.e., the cotyledons are entirely pigmented and the intercotyledonary areas are free from pigment.

In the foetal lamb the pigment cells are distributed throughout the mucosa (Fig. 17).

During pregnancy, the pigment seems, on superficial examination, to disappear, but microscopical examination has shown that a few cells remain in the intercotyledonary areas. The number of such cells is quite insufficient to account for the reappearance of the pigment during the following anoestrum/

anoestrum however. The source of the new cells has not been determined. The pigmentation of the Fallopian tubes is unaffected by pregnancy.

The occurrence of pigmented cells in the uteri of immature and even foetal animals, is in itself sufficient indication that the pigment is not of haematogenous origin. It has already been point-:ed out that the cells are structurally almost identical with the melanoblasts of the skin, and of the lamina fusca of the eye: such differences of structure as are present may reasonably be ascribed to the differences in the nature of the connective tissue that the cells occupy.

The pigment granules are very small and of practically uniform size. They are of a deep brown colour. The pigment was found to be insoluble in strong sulphuric acid but probably slightly soluble in strong caustic soda. Iron could not be detected by the ferricyanide reaction either in sections of pigmented tissue or in the ash of pigmented mucosae. The pigment was bleached by hydrogen peroxide and by chlorine. These reactions are all characteristic of melanin.

Finally, it has been found that the uteri of Shetland ewes are pigmented reddish brown like the skin and wool, not black as in other breeds.

There can be no doubt therefore that the characteristic/

characteristic pigmentation of the sheep's uterine mucosa is due to the presence of true melanoblasts and is in no way related to blood extravasation as was supposed by Bonnet, Kazzander and Marshall.

It is a matter of some difficulty to con-:jecture as to what possible functional significance the uterine pigment may possess. During pregnancy the pigment cells are very largely broken down and the pigment granules engulfed by the phagocytic cells of the foetal trophoblast. The pigment might conceivably be used by the foetus but the highly insoluble nature of melanin renders the validity of this interpretation very improbable. Pigment cells of identical nature have been found in other abdominal viscera, notably in the capsules of the suprarenal glands and in the walls of the pelvic arteries. They are quite commonly found between the tubuli of the epididymis in the male. It seems probable therefore that the occurrence of this pig-:ment is purely adventitious and that it serves no function.

It is of interest to note that heavy pig-:mentation of the uterus is found in breeds of sheep such as the Cheviot and Border Leicester, in which skin pigmentation has been almost entirely eliminated by selection.

Pigmented/

Pigmented cells of another type are found in the uterine mucosae of ewes which have borne lambs. They are particularly common during the first 3 to 4 months after parturition. These cells are round or oval in shape without processes, and of a uniform pale or deep brown colour, that is, the pigment is non-granular. The nucleus is very small and pycnotic, and these cells may be dead. They are distributed throughout the mucosa but are particular-:ly common in the deep tissue of the cotyledons.

These cells seem to be homologous with the pigmented cells found in the uteri of mice, rats, etc. after pregnancy, which have been described by Benazzi (17, 18) and others. The circumstance that they occur only after parturition suggests that their pigment is of haematogenous origin. Verne and Isidor (163) have described branched pigment cells in the uterus of the human. The relation of these to the pigment cells of the ewe is uncertain.

IX : CYCLICAL CHANGES IN THE VAGINA.

1. THE GROSS ANATOMY AND HISTOLOGY OF THE VAGINA.

The general anatomical andhistological features of the vagina and vulva of the sheep have been described by Schmaltz (144), Ellenberger and Baum (52) and others; it will suffice for the present purpose, therefore, to recall only the main structural features and to devote some attention to those parts which are subject to structural alterations in accordance with the physiological state of the animal.

The vagina, in a mature animal, is about 6 cms. long and 2 to 3 cms. in diameter: normally, its walls are collapsed and apposed to one another. The vestibule (i.e., that part of the vagina lying posterior to the urethral opening and derived from the urogenital sinus of the embryo) is about 4 cms. long and of slightly less diameter than the vagina proper. The clitoris lies just within the vestibule: superficially it is only about 2 mm., long. The fossa clitoridis and the fossa urethralia are both poorly developed. The mucosa of the vagina usually bears a great number of irregular longitudinal folds, but that of the vestibule is nearly always/

always smooth. The surface of the vaginal mucosa often bears a number of small glassy projections 2 to 3 mm. in diameter ("lymph follicles"). These are due to subepithelial accummulations of lymph. They are most frequently seen during the first month after parturition: they are rarely present in virgin sheep.

The epithelium of the vagina and vestibule is entirely of one type, i.e., stratified squamous epithelium (see Fig. 30). True secretory cells are entirely absent. The thickness of the epithelium in all parts of the vagina is subject to considerable variation but there are no constant regional differences.

The average number of cell layers is 5 to 6 but the number may be as small as 2 or as large as 12 or more. Localised thickenings or papillae of the epithelium projecting into the stroma are common: in these regions there may be 20 to 25 layers of cells.

The basal layer or stratum germinativum of the epithelium is composed of tall columnar cells with large, oval, reticulate nuclei. The inter-:mediate layers are composed of cells of more polygon-:al form but of similar structure. These cells have normally very well defined cell walls. Intercellular canals traversed by fine protoplasmic "bridges" are present but they are not often visible except in badly/

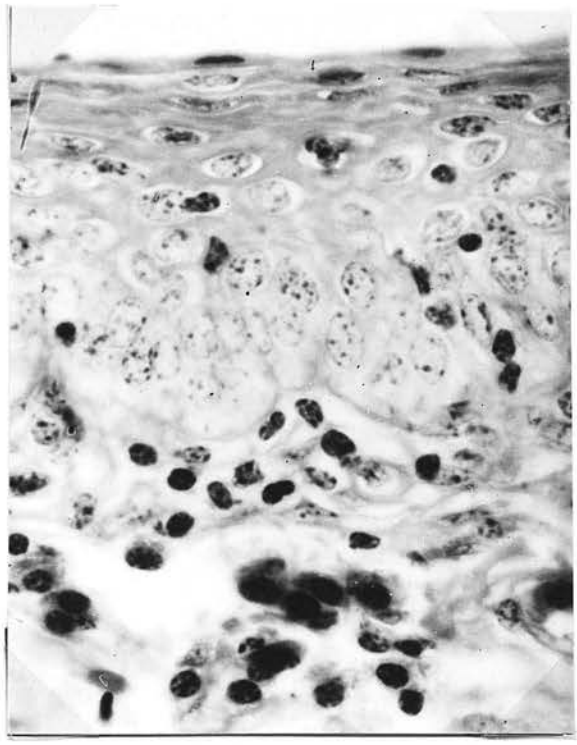


Fig. 30. Section through superficial vaginal mucosa. x 800

badly fixed material, in which shrinkage of the cells has taken place. The character of the surface layers varies enormously but, in general, they are composed of flattened cells, with shrunken nuclei and either autolysed or keratinised cytoplasm.

Sometimes, a membrana propria separates the epithelium from the underlying connective tissue but this never appears to be complete over any considerable area and is usually not demonstrable at all. Quite frequently the epithelium merges into the connective tissue. Superficially, the wall of the vagina is composed entirely of collagenous fibrous tissue: in the deeper parts there are bundles of smooth muscle. The number and arrange-:ment of these muscle bundles vary in different parts of the vagina but they are divisible roughly into an internal circular coat and an external longitudinal coat. It must not be supposed, however, that the muscles form definite sheets as they do in the uterus: they occur as isolated bundles separated by considerable amounts of connective tissue. Striated muscle has not been found in the sheep's vagina. External to the muscle layers is a further coat of loose connective tissue in which are contained the large blood vessels of the vagina. This layer also contains a considerable amount of fat and occasional nerve ganglia. The relations of these ganglia have/

have not been traced out. The peritoneum is reflected about 2 inches behind the os externum of the cervix. The wall of the vestibule, although composed of the same elements as that of the vagina, is much thinner.

The vestibule is more richly vascular than the vagina and its capillaries come closer to the epithelium: it thus always seems flushed in comparison with the vagina. Small amounts of erectile tissue are found in the vestibule but not in the vagina.

Lymphocytes are scattered profusely throughout the connective tissue of the vaginal and vestibular walls: they are concentrated, however, immediately beneath the epithelium, the basal layers of which they penetrate in considerable numbers. In the vestibule there are many small lymph nodes lying immediately beneath the epithelium, especially near the clitoris. Over these nodes and also where there are unusually heavy accumulations of lympho-:cytes, the epithelium is commonly disorganised and the lymphocytes penetrate to the vaginal lumen (see Figs. 31 and 32). Where the epithelium is intact, lymphocytes appear to be unable to penetrate to the surface, or to survive being carried through by the growth of the epithelium, for those that occur in the superficial layers are invariably degenerate./

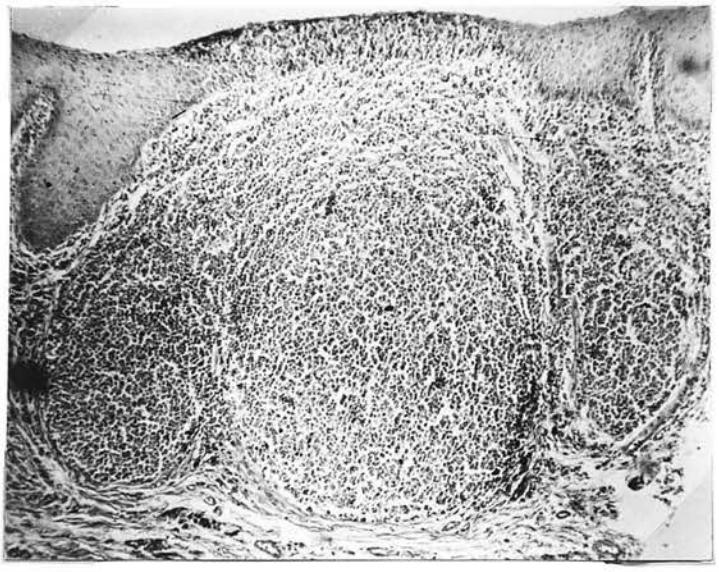


Fig. 31. Showing a vestibular lymph node, and the destruction of the overlying epithelium. Stained with Heidenhain's haematoxylin. x 90.

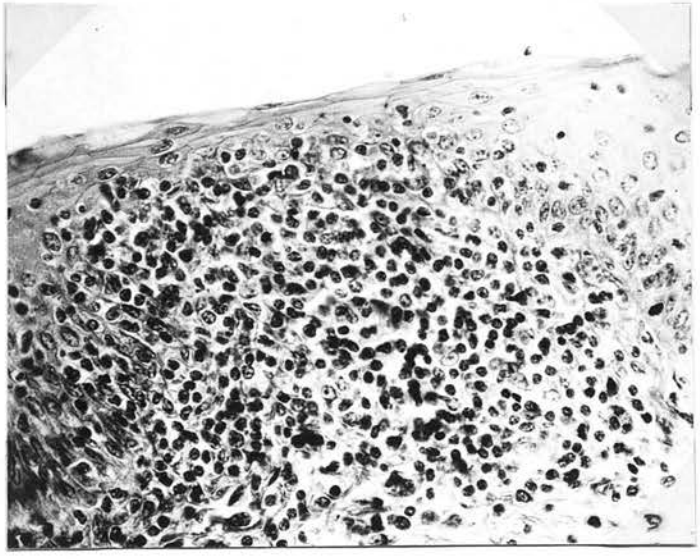


Fig. 32. Section through vaginal mucosa in the region of a heavy accumulation of lympho-:cytes. The epithelium above is partially destroyed. Stained with Heidenhain's haemat-:oxylin, differentiated in Van Gieson's stain. x 330.

degenerate.

The part played by the lymphocytes of the loose connective tissue is still a matter for speculation, and it is not intended to discuss in this place the relative merits of the various hypotheses that have been propounded with regard to them: their distribution in the sheep's vagina does suggest, however, that they may play a part in the defence of the vagina against bacterial invasion. This view gains some slight support from the circumstance that polymorphonuclear leucocytes are ordinarily absent from the vaginal mucosa. Such barrier as these may present against infection in the rat, guinea-pig, etc., is thus absent in the sheep.

Neutrophilic polymorphs occurred in great numbers in the vaginal mucosae of all the experimental ewes but in only a very few of those from non-experimental sources, and always in low concentrations. They were found in vaginal sections from only a small proportion of the slaughter-house ewes showing polymorphs in the vaginal smear. Leucocytic infiltration of the vagina must therefore be very localised, for polymorphs do not occur in higher parts of the generative tract. They were found in the superficial stroma and throughout the epithelium but were concentrated in the superficial layers of the latter. It thus appears probable that polymorphs penetrate/

penetrate the epithelium rapidly and through their own activity but do not pass into the vaginal lumen readily. Lymphocytes on the other hand are probably carried almost passively through the epithelium by its growth and dehiscence. They are unable to sur-:vive the passage of the epithelium and very rarely reach the vaginal lumen in a recognisable form; they are thus rarely found in the vaginal smear.

It has been suggested that the highly lobed condition of the nuclei of the polymorphs found in vaginal smears, not only from sheep but from rats, etc., is acquired during the passage through the epithelium. This is not the case in the sheep; the polymorphs of the stroma are as highly lobed as those of the superficial epithelium.

Extensive accumulations of lymphocytes cause disorganisation of the epithelium but individ-:ual lymphocytes have no discernible effect. The heavy infiltrations of polymorphs found in experi-:mental ewes caused no apparent disorganisation except in the superficial, dead layers of the epithelium.

Eosinophilic granular leucocytes occurred in the vaginal mucosae of a few animals.

The glandulae vestibulares majores (glandulae Bartholini) which are regularly well-developed and probably functional glands in the cow, are represented in the sheep by diffuse, very rudimentary/

rudimentary glands, the state of development of which varies considerably from one animal to another, (see Fig. 33). They are composed of large clear cells with distinctly reticulate cytoplasm . The deeper glands have commomly no lumina. The connective tissue theca of the glands is frequently pigmented. They are seldom demonstrable except in histological preparations and sometimes appear to be completely absent. It is very improbable, there-:fore, that these glands have any physiological function in the ewe.

It has already been mentioned that the superficial part of the clitoris is very slightly developed. The corpus clitoridis is also very small in comparison with that of the other domestic mammals. It is usually a club-like body about 2 cms. long, and 0.5 cms. in diameter: occasionally the anterior end is bifurcated as in the cow. The fossa clitoridis in lined by an epithelium similar to that of the vestibule. The epithelium is continu-:ed as a solid invagination round the corpus clitor-:idis. This epithelium as well as that of the clitoris proper, is frequently deeply pigmented even in white-skinned sheep.

2. GROSS CHANGES.

During the oestrous cycle the vagina under-:goes marked alterations. Some of these changes are/

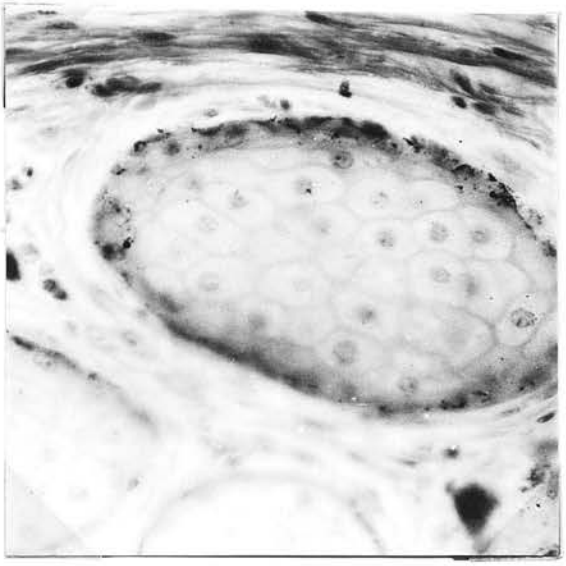


Fig. 33. Section through one of the lobes of Bartholin's glands, in a ewe in which these glands were well developed. The theca of the glands is pigmented.

are sufficiently great to be observable in the living animal by examination from the vulva, or at autopsy by superficial examination of the vaginal mucosa.

At the onset of heat, or immediately preceding its onset, there is a considerable increase in the vascularity of the vaginal and vestibular mucosae, coinciding with the congestion of the vulva to which reference has already been made. To naked-eye examination this congestion appears to affect the vestibule much more than the vagina, but it is doubtful whether the blood vessels of the two actually differ in their reaction at this time: the greater vascularity of the vestibule and the thinness of its wall probably makes the changes in the vestibule more obvious. The congestion of the vagina comes and goes at the same time as that of the vulva and is subject to the same variations. Congestion is accompanied by oedematous swelling, especially of the vestibule. This oedema is slight in the unmated animal but is greatly accentuated by coitus.

During heat the vaginal muscles relax considerably: this loss of tonus is usually of shorter duration than the heat period. A similar change in the vestibular muscles of the sow was observed by McKenzie (111).

In anoestrum and dioestrum the surface of the vaginal mucosa, like that of other mucous

membranes, is moist and pink. With the onset of heat, the quantity of mucus in the vagina increases greatly: towards the end of heat this mucus rapidly disappears, probably entirely through expulsion from the vulva, and it is replaced slowly by a thick layer of yellow caseous material. This material becomes most dense near the cervix but extends throughout the vagina and vestibule. At this time the mucosal surface is dry and sticky. The accumulation of caseous material reaches its height approximately 24 hours after the end of heat, and thereafter the material slowly liquifies and disappears.

The constitution of the vaginal secretions and the precise time relationships of the changes in their character to the oestrous cycle have been studied in detail and may now be dealt with in full.

3. CHANGES IN THE VAGINAL SECRETIONS.

Introduction.

Since the present investigation was commenced a number of short accounts of the vaginal changes in the ewe during the dioestrous cycle have been published. Darlow and Hawkins (47) have given a brief account of the changes in the constitution of the vaginal smear in Merino ewes. Smears were taken at 24 hour intervals. Cole and Miller (40, 42) have described the smear changes in somewhat greater/

greater detail, and in the last paper mentioned, refer to the histology of the vaginal wall. Casida and McKenzie (37) described the histology of one area of the vagina in nine ewes autopsied at differ-:ent intervals after the commencement of heat.

While there is agreement between the observations of the authors mentioned and also with those of the present author as to the main features of the vaginal cycle, there are marked differences in detail. Thus Cole and Miller found that leucocytes may be present in the smear at any time during the cycle but that there is a peak, lasting about a day, between the third and the eighth days. From the account given by Darlow and Hawkins there would appear to be two peaks in the leucocyte curve, one about the eighth day of the cycle and the other at the fourteenth or fifteenth day. Casida and McKenzie found that the number of leucocytes was greatest during the latter half of dioestrum. Thus there is only slight agreement between the different observers as to the time of greatest leucocytic infiltration. Cole and Miller, and Darlow and Hawkins do not distinguish between the actual number of leucocytes traversing the vaginal mucosa and the relative proportion of leucocytes and other cell-types in the vaginal smear: Casida and McKenzie's data refer to histological sections of the vaginal mucosa and therefore refer to the actual number of leucocytes/

leucocytes in the epithelium without reference to the number which reach the vaginal lumen.

It may be noted also, that no attention has been paid to the possible influence of smearing upon the normal cycle of events in the vagina. Cole and Miller, and Darlow and Hawkins used only those animals which were used daily for smearing.

The bearing of the present author's work upon the results of other authors will be dealt with more fully as the individual changes are described.

A. Changes in Appearance and Consistency.

The changes in the appearance and consis-:tency of the sample of vaginal contents are simply a reflection of the changes in the macroscopic appearance of the vaginal mucosa to which reference has already been made.

During the early part of the heat period the sampling rod brings away from the vagina copious quantities of clear, frothy, watery mucus. Some-:times the mucus is flecked with white, or yellowish spots. Towards the end of heat the amount of mucus usually decreases considerably: at the same time its consistency and appearance change, and it gradually becomes cloudy and yellowish white in colour. These changes continue until about 24 hours after heat at about which time they reach a peak.

The/

The sample of vaginal contents removed at this time is a mass of thick cheesy material, sometimes white but more frequently yellowish in colour. Rarely it is dry and almost powdery: rarely again, it is more creamy. As the cycle progresses the sample becomes at the same time both less abundant and of more liquid consistency. By about three days after the end of heat the smear is ordinarily a homogenous yellow creamy substance. By about the fifth day after heat a state of relative stability is reached which persists, practically without change, until the onset of the new heat period. During this relatively long and monotonous period, which may be termed the "resting phase" of the vagina, the sample of vaginal contents is subject to irregular but slight changes. In general, however, it is a rather sparse, distinct-:ly yellow, cream. The depth of "yellowness" of the vaginal sample may be said to increase from the time of maximum density of the smear until the mid-cycle.

B. Changes in total Mucus and in total Free-cell Contents.

Microscopical examination of the vaginal samples has shown that they may be analysed into two main components, (a) mucus and (b) cells and products of cell disintegration. It has been determined, also,/

also, that the consistency and gross appearance of the vaginal smear is a very good indication of the relative preponderance of these two components. The "clear mucus" smear consists almost exclusively of mucus; cells are almost completely absent. The dry, cheesy smear usually found about 24 hours after heat consists almost exclusively of cells: the "creamy" smear of the mid-cycle consists of an admixture of cells and mucus, the relative amounts varying according to consistency. Consistency is also affected, however, by the nature of the mucus in which the cells are suspended.

Since the vaginal smear may be thus readily analysed into its main components and since, also, it has been shown that the mucus and cells of the vaginal smear represent phases of activity of two different parts of the generative tract, cervix and vagina respectively, it has been considered desirable to treat these two components as separate entities. The graphical records plotted for each ewe, some of which are reproduced in the Appendix to this Thesis, represent the result of this treatment: these graphs are intended to show the variation in absolute amount, by volume, of mucus and cells in the vagina during the entire breeding season of each ewe, or until such time as they were autopsied, or became pregnant.

Before/

Before the results obtained are discussed, however, it is necessary to give some account of the methods employed in arriving at the figures recorded in the graphs.

i) Methods of estimation.

The changes in the total volume of free vaginal contents are sufficiently large to allow relative estimates of volume to be made easily and with a considerable degree of accuracy. At the time of sampling, each smear was placed in one of ten arbitrary grades of abundance, referred to as "very sparse", "sparse", etc. The difference between grades was by no means equal, however, since small changes in volume are clearly more conspicuous when the absolute volume is small than when it is large. Determinations of the approximate actual amounts represented by each of the ten grades were made at the time of autopsy of experimental ewes. Each figure for smear volume estimated at the time of smearing was then corrected in accordance with the actual values determined at autopsy. In this way figures were obtained which represent approximately the actual volume changes of free vaginal contents in each ewe. The actual volume of a "Grade 10" (the highest point reached) smear is about 50 to 60 c.cs. A change of one point in the graph represents therefore about 5 to 6 c.cs.

The/

The consistency and appearance of the vaginal sample formed the basis of the estimates of relative cell and mucus contents. Each smear, as taken, was placed in one or other of ten grades of consistency: at the same time a note of the appearance and "texture" of the smear was taken, since consistency depends not only upon the per-:centage of cells present but also upon the fluidity of the suspending mucus, which changes considerably. Typical samples of each grade were centrifuged in graduated tubes (this was not possible with the very richly cellular samples) and the relative proportions by volume of cells and mucus determined. It may be noted that the same volume of cells has about the same effect on smear consistency irrespective of the type of cell predominating, epithelial cells or leucocytes.

Each corrected figure for total smear volume was then divided into its component parts, cells and mucus, according to the relative proportion indicated by its consistency and appearance. It is the figures arrived at in this way that are plotted in the graphical records reproduced in the Appendix.

It is apparent that the methods employed in making these estimates were very approximate: it is important to recognise therefore that only the major and recurring variations in the graphs can be regarded as significant. The minor variations, except when they appear regularly, cannot be inter-

interpreted as significant without very careful consideration of the foundations upon which they are based. For example the mucus graph usually shows a secondary rise in metoestrum. It is by no means certain that this rise is actually due to an increase in mucus secretion. The preceding depression of the mucus content may rather be apparent and due to the absorption of mucus in the multitude of cells which is present in the vagina at this time.

The Mean Graphical Record of Vaginal Smear Changes for all Experimental Ewes reproduced in the Appendix was constructed from the graphs for individ-:ual ewes in the following manner. Starting with the second day preceding that on which oestrous commenced in each cycle, the mean daily values of "total cells", "total mucus", and "vulvar congestion" were read off from the graphs for the succeeding 17 days. The figures for the 65 normal cycles were then summated and the mean values for each day of the cycle deduced: these values are plotted as the mean smear record. Since the heat period might commence at any time on the third day of the individual cycles, the figures plotted are mean values for the first, second, third, etc., 24 hour periods after the commencement of heat. The duration of oestrum plotted is the mean value, 36 hours.

It is obvious that this graph is in no sense a representative graph, typical of the normal cycle./

cycle. It is intended to show, not the characteris-:tic form of the graphs but the periods, with relation to the commencement of oestrum, over which the mucus or cell content of the vagina and the vascularity of the vulva may be expected to rise above their normal dioestrous level.

ii) Changes in the total mucus content.

The general relationships of the vaginal mucus content to the oestrous cycle have already been indicated. Mucus is never entirely absent from the vagina but the amount present increases enormously at the time of heat. The amount ordinarily present during dioestrum is about 0.5 to 2 c.cs. During the oestrous period the content normally rises to 30 to 40 c.cs. and sometimes to as much as 60 to 70 c.cs.

The time relationship between mucus secretion and oestrum.

As the secretion of mucus is clearly very closely associated with the mating phase of the cycle it is important to consider the precise time relation-:ship between the two since this may be expected to throw light upon the physiological relationship involved.

Reference to the individual graphical records shows that the time of commencement of the mucus flow with reference to the onset of heat varies considerably; usually, however, the mucus appears in the/

the vagina several hours before heat begins. The extremes appear to lie at about 24 hours before and 8 hours after the onset of heat. Since the mucus is not a product of the vaginal mucosa but of the cervix, the time of its appearance in the vagina is probably some hours later than the commencement of secretory changes in the cervix. Thus mucus secretion should be regarded as a phenomenon slightly preceding rather than accompanying heat.

While an increase in vaginal mucus content usually takes place before heat beings the maximum concentration is very seldom reached until heat is well advanced. The phase of mucus secretion is normally very short: by the end of heat the mucus content is very seldom above its normal dioestrous level.

In a few cases samples of vaginal contents were taken at short intervals throughout the heat period: the records of these cases show that the mucus content during oestrum does not rise steadily to a peak and then steadily decline but that it fluctuates widely and rapidly. The content may fall from 50 c.cs. to 5 c.cs. in the course of half an hour and rise again to its original value almost as quickly. The probable explanation of these rapid fluctuations is to be found in the forcible expulsion of mucus from the vulva to which reference has already been/

been made.

It will be noticed that in a number of cases no rise in the mucus graph at heat is recorded. It appears probable that in these cases, had obser-:vations been made more frequently, a marked rise would have been found: it probably so happened that the samples were taken shortly after the expulsion of mucus.

While a sharp rise in the mucus graph is thus generally indicative of the early part of the heat period, the time relationship between the two is not sufficiently close to enable the phases of the heat period to be distinguished from one another by the mucus content of the vagina alone. It is certainly not possible to distinguish between pro-oestrum and oestrum on the basis of mucus content as has been suggested by Cole and Miller (42).

Changes in mucus content during interoestrum.

In a majority of cases the mucus graph remains at a uniformly low level from the end of one heat period to the commencement of the next, but in some cases considerable increases in mucus content occur during interoestrum. In no case, however, did one of these peaks rise to the level that is usual in oestrum. These sporadic rises in mucus content could not be confused with the normal peaks of oestrum, since not only the volume but the consistency/

consistency and appearance of the secretions were always different.

The mean graph for all ewes shows that these peaks are of truly sporadic appearance, i.e., they do not appear more frequently at any one stage of the cycle. A possible exception is the peak occurring immediately after oestrum, to which reference has been made. Cases do occur where this secondary rise in mucus secretion is undoubtedly real: but in the majority of cases where an apparent depression in the mucus curve occurs immediately after heat, this depression corresponds in time to a great increase in the cell content of the vagina. It appears probable therefore that the depression in the mucus curve is apparent and due to the absorption of mucus in the mass of free cells. On the basis of this interpretation the secondary wave of mucus secretion is artificial and due to the removal of cells from the vagina.

iii) Changes in the character and pH of the vaginal mucus.

The abundant mucus found in the vagina during heat is uniformly fluid and serous in consis-:tency. It is normally frothy in the vagina and, when a small quantity of it is shaken up in a test tube, a relatively permanent froth is formed.

The small quantity of mucus present in the vagina during interoestrum is ordinarily much more tenacious/

tenacious and "stringy", i.e., it is not of uniform consistency. When the mucus content rises to a considerable value in interoestrum, as it may sometimes do, the consistency of the mucus is usually intermediate between that characteristic of oestrum and that characteristic of interoestrum. The thin frothy mucus characteristic of oestrum has not been observed outside that period.

Accurate determinations of the pH of the vaginal mucus have not been made. Rough determinations by means of indicators show the fluid mucus of oestrum to be well on the alkaline side - about pH 8.4. A rather lower value was indicated for the "stringy" mucus of dioestrum.

iv) Changes in the total free-cell content.

Although they are subject to considerable variation and to frequent exceptions the changes in the total free-cell content of the vagina are a great deal more regular in their appearance than those of the mucus content. Changes take place more slowly and the forcible expulsionsof vaginal contents which so greatly affect the mucus graphs have practically no effect on the cell content since they take place only during oestrum when the cell content is very low: samples taken at relatively wide intervals therefore give a very fair picture of the changes which actually take place.

As in the case of mucus, there is only one characteristic/

characteristic alteration in the total free-cell content of the vagina during the oestrous cycle, a marked rise immediately following heat. The in-:crease usually commences during the latter half of oestrum but sometimes not until after heat has ceased. A peak is reached after about 24 to 48 hours, the peak nearly always coming after the cessation of heat but occasionally within the heat period. The peak value for free-cell volume is about 35 to 40 c.cs. in normal cases. In many cases the "peak" tends to become a plateau, that is, there is a tendency for the cell content to remain at a high level for 24 hours or more: more frequently the cell content commences to fall away rapidly soon after the peak is reached, and by the following day the content is considerably lower. The time taken to reach the normal dioestrous level varies from as little as one day to as much as ten days: in most cases the time occupied is 2 to 3 days. In some cases it appeared that the delayed disappearance of cells from the vagina might be due to the small amount of mucus present, since mucus undoubtedly assists in the expulsion of the mass of dead cells from the vagina. This factor was not sufficient to explain all the cases of delay, however. There is probably a relation between the length of oestrus and the persistence of high cell content: ewe 701 which had characteristically/

characteristically long oestrous periods also showed very wide peaks in the total cell graph, indicating continued desquamation from the vaginal epithelium. An exceptionally short oestrous period did not seem to influence either the duration or the intensity of desquamation however.

No part of the characteristic total cell graph is entirely constant in its relation to any part of the oestrous period. The closest relation-:ship seems to be between the commencement of heat and the first increase in the free-cell content, which usually follows the onset of heat after about 24 hours.

The vaginal changes at the sixth oestrous period in experimental ewe 816 deserve particular attention. It will be seen from the records that in this case the vaginal changes occurred at the normal time, i.e., 16 days after the previous cycle and 16 days before the subsequent cycle. Oestrum, however, was delayed, for it occurred 22 days after the previous heat and 12 days before the subsequent heat. Thus, in this peculiar case, both the mucus flow and the desquamation of the vaginal epithelium were pro-oestrous phenomena. The significance of this case will be discussed elsewhere.

C./

C. Changes in Total Mucus and Total Free-Cell Contents in non-experimental Animals.

The changes described in the foregoing pages refer exclusively to the smears taken from the experimental ewes. The results deduced from control material were entirely corroboratory and may be presented much more briefly.

Of the samples taken from non-experimental ewes at the Institute 12 showed a mucus content above "Grade 5". All these ewes were on heat at the time of smearing. In 5 other ewes which were found to be on heat at the time of smearing the mucus and cell contents were both low. Eighteen smears were dry and cheesy and indicated a total cell content above "Grade 5". The remaining smears all indicated mucus and cell contents of less than "Grade 5" and mostly less than "Grade 2". The proportions of cells and mucus in them varied from one extreme to the other. Thus in these control ewes all types of smear encountered in the experimental ewes were met with, with the exception of the very abundant "creamy" smears sometimes encountered in the experimental animals (smears with high mucus and high cell contents). The smears from the ewes on heat were of exactly the same type as those encountered in the experimental ewes during oestrum and the time of subsequent/

subsequent heat in those ewes which gave richly cellular smears indicated that they had all been on heat during the 2 or 3 days preceding the day of sampling. Richly cellular smears occurred in the experimental ewes during the three days following the end of heat.

In the case of material collected at the slaughter houses, it was, of course, not possible to determine either the psychological state of the animal at the time of slaughter or the date of the last heat period. The stage of the oestrous cycle at the time of killing could, however, be determined with considerable accuracy from the state of the ovaries; the manner in which this was done has already been explained. Records of the volume and nature of the vaginal contents were made for nearly 700 ewes killed during the dioestrous cycle: the mean values for total mucus and total free-cell con-:tent, estimated in the same manner as with the experimental ewe material, are given in Table VII together with the mean values for the same periods read off from the mean record graph of the experi-:mental ewes. It will be seen that there is very close agreement between the two independent sets of data. That the gross changes in the vaginal smear of the epxerimental animal do reflect normal conditions very closely may not, therefore, be doubted.

Very/

TABLE VII.

Mean Values for Total Mucus and Total Free-Cell Content of the Vagina.

Ovarian Class.	Slaughter House Values.		Exp. Ewe Values.	
	Mucus	Cells	Mucus	Cells
III.	4.7	.4	4.5	1.1
IV.	2.6	2.0	2.0	3.25
V.	1.6	3.5	1.25	2.8
VI.	1.2	0.4	1.0	0.8
VII.	1.3	0.22	3.0	0.6

Very high total cell content values were encountered in the slaughter house material in Stages IV and V only, and high mucus values only dur-:ing Stage III (excluding pregnant animals).

It will be noted, however, that the slaughter house data appear to lag behind the experi-:mental slightly, i.e., alterations appear earlier in the experimental ewe columns than in the slaughter-house ewe columns. This tendency is exhibited also in other comparisons between the two sets of material and it can have only one reasonable explanation, i.e., the time of ovulation, taken on the basis of publish-:ed observations, to be about 30 hours after the commencement of heat, has been placed too late in oestrum.

A further discrepancy between the two sets of figures lies in the circumstance that the experi-:mental ewe figures for total cell content are consistently above the corresponding figures for the control ewes. The discussion of the probable cause of this discrepancy is deferred until the microscopi-:cal characters of the smears have been described.

D. <u>The Cellular Components of the Vaginal Smear and their Relation to the Dioestrous Cycle</u>.

So far, reference has been made only to the total amount of free cellular material present in the vagina/

vagina during different phases of the dioestrous cycle, without reference to the type of cells present. In most of the other mammals that have been studied very distinct alterations in the representation of the various cell types present in the vagina have been found to take place during the dioestrous cycle, and in the sheep, Darlow and Hawkins (47), Cole and Miller (40, 42), and Casida and McKenzie (37) have all described changes resembl-:ing those described for other mammals. It has been pointed out, however, that the accounts given by these authors differ considerably from one another and it is pertinent to present in detail the findings of the present investigation, since the material upon which it was based was both more extensive and more adequately controlled than was the case in previous investigations.

The morphologically recognisable elements which have been found at one time or another in the vaginal smears of either experimental or slaughter house ewes are as follows:-

1. Vaginal epithelial cells: healthy, degenerate and cornified.
2. Leucocytes; a) neutrophilic polymorphonuclear.
   b) eosinophilic.
3. Lymphocytes.
4. Uterine epithelial cells - always degenerate.

5./

5. Cervical epithelial cells.
6. Bacteria.
7. Erythrocytes.
8. Isolated nuclei of vaginal epithelial cells.
9. Cell detritis of several types, referable to one or other of the above.

But of these only vaginal epithelial cells and neutrophilic polymorphonuclear leucocytes were found with any regularity and epithelial cells alone were present in all smears. Uterine epithelial cells and cervical epithelial cells have been identified in only a very few smears and their presence appears to have no relation to the physiol-:ogical state of the animal. Erythrocytes were not found in any of the smears taken from living ewes nor have they been found by other authors, but they were found in a great number of smears from post-mortem material: undoubtedly the erythrocytes arose, in most cases, by contamination from surfaces cut in butchering the ewes but in a few ewes killed in metoestrum erythrocytes were found so intimately mixed with the vaginal contents that they could scarcely have originated by contamination. It has been noted that slight haemorrhage sometimes takes place in the uterus during oestrum: the possibility that erythrocytes may be an occasional component of the oestrous and metoestrous smears may not, therefore, be/

be eliminated, although no definite relation between uterine haemorrhage and vaginal erythrocytes was established. Such erythrocytes as may be present in the vagina must be derived from the higher parts of the reproductive tract since vaginal haemorrhage does not take place.

Bacteria occurred at one time or another in the vaginae of all the experimental ewes and in some of them were almost constantly present. The bacteria found were almost exclusively streptococci. They were found also, but rarely, in the smears from non-experimental ewes and in those taken from slaughter-house material. Their occurrence is thus clearly due to chance infection. That they occurred regularly in all the experimental ewes indicates that the simple antiseptic precautions adopted in smearing were inadequate to prevent infection entirely. Once introduced, the bacteria seemed to thrive in the environment of the vagina, but there is no evidence to suggest that they affected the normal changes in the vagina in the least, nor did they ever reach such a concentration that they could have affected the estimate of total cell content appreciably. In the experimental ewes the number of bacteria changed considerably as the environment became more or less favourable. They reached their highest concentra-:tions during the periods of liquefaction of the epithelial/

epithelial cell masses, i.e., about 3 to 6 days after heat. They were greatly reduced in number during oestrum and most of dioestrum, and they were frequent-:ly almost annihilated by heavy infiltrations of healthy leucocytes. Certain ewes seemed to present a more favourable medium than others: thus in ewe 773 they were nearly always present in high con-:centrations while in ewe 816 they appeared only occasionally and in small numbers. The reasons for this difference in susceptibility were not determin-:ed: the intensity of leucocytosis was not the controlling factor. Hypothetically, the difference may be ascribed to the pH of the cervical mucus since, apparently, the fluid mucus of oestrum is more alkaline than the stringy mucus of dioestrum, and the bacterial count is greatly reduced in oestrum.

Cell detritus in the vaginal smears could be divided roughly into two types, that derived from epithelial cells and that derived from leucocytes. Epithelial cells give rise to granular detritus, usually unstained. Free epithelial cell nuclei, more or less distorted and autolysed are another product of epithelial cell degeneration. Neutro-:philic leucocytes, on the other hand give rise to a very distinctive "stringy" type of detritus, staining an intense purple with Giemsa (see Fig. 36). This stringy detritus is derived from the nucleus alone: the/

the cytoplasm forms a granular detritus like that of the epithelial cells.

i) Epithelial cells.

The epithelial cells of the vaginal smear show a considerable range of variability in both morphology and staining reaction. There is no constant regional differentiation of the vaginal mucosa, however, and all the forms found represent the changes which may take place in any vaginal epithelial cell from the time of its production by the stratum germinativum to the time of its desqua-:mation from the epithelium. While a complete range of transitional forms exists it is convenient to distinguish four main types of epithelial cell:

1) Small healthy nucleated cells measuring about 15 μ in diameter. These occur either singly or in sheets of up to 50 cells. They have small reticul-:ate nuclei staining deep purple with Giemsa. The cytoplasm, of which there is relatively little, is clear and free from granules: it either fails to stain at all or stains blue (basophilic) with Giemsa. Small vacuoles are sometimes present. The cytoplasm is perfectly labile so that, although the cells have a distinctive, angular shape when adherent to one another in sheet form, when isolated they round off and adhere to the glass slide. This type of cell was encountered in anoestrum only. Mitoses have not been/

been observed in this or in any other type of cell found in the vaginal smear.

2) Large nucleated cells about 35 μ in diameter (Fig. 34). This type is simply a much larger cell of the same structure as the first type. The nucleus is larger and of somewhat looser texture. The reticulate character and the staining reaction remain unchanged. The increase in the cytoplasmic volume is relatively greater than that of the nucleus. The cytoplasm has less affinity for stain and tends to stain purple (neutrophilic) rather than blue. Vacuoles are more common and larger than in the above type; their presence frequently distorts the nucleus. Many cells of the type are found with no trace of vacuolisation, however. The nature of the vacuoles is uncertain: they have no affinity for stains. A relatively fluid consistency is indicated by their tendency to run together. Neu-:trophilic granulation of these cells has been observed but is rare.

With regard to cytoplasmic lability these cells occupy a position intermediate between the first type and the following. They have little tendency to adhere to one another and usually occur isolated in the smears. In many cases the isolated cells round off as in the case of the small cells; in other cases the cells retain an angular form sugges-:tive of the next type. Multinucleated cells of this/

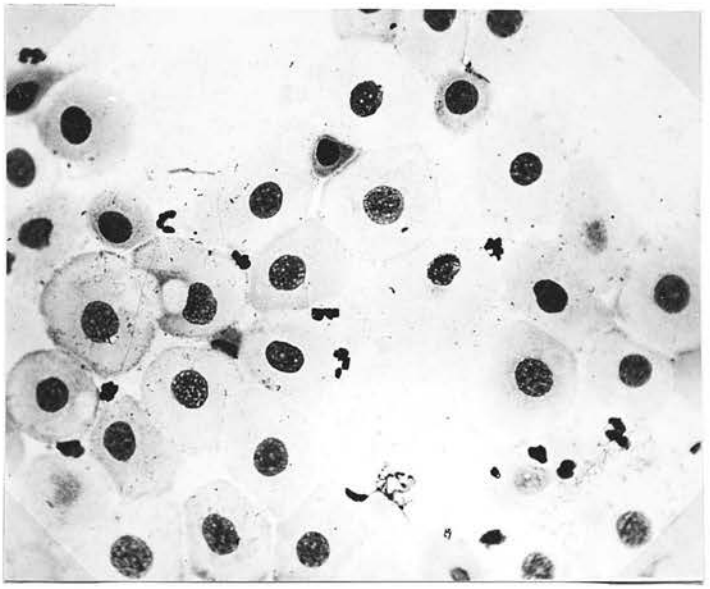


Fig. 34. Second type of superficial vaginal epithelial cell - the healthy nucleated epithelial cell. x 400.

Fig. 35. Third type of superficial vaginal epithelial cell - semi-cornified type. Note granulation of some of the cells. x 400.

this class occur, cells with five or six nuclei having been encountered.

This type of cell may be designated the characteristic cell type of the superficial vaginal mucosa since it is very rarely entirely absent from the smear and is the only type ordinarily found dur-:ing anoestrum. It is strictly comparable to the superficial cell of other stratified mucous membranes such as that of the mouth.

These cells represent the culmination of healthy growth in the vaginal epithelium: the cells have lost their connection with one another and are so far removed from the blood stream as to be in-:capable of further growth. The form which their subsequent death and degeneration takes is, however, not always the same but may take one of two forms, (a) autolysis and liquefaction, (b) keratinisation (cornification), that is, the transformation of the cell body more or less completely into keratin. Degeneration of the first type gives rise to dis-:integrated forms of the nucleated epithelial cell and ultimately to cell detritus: degeneration of the second type gives rise to the third characteristic vaginal epithelial cell type, i.e., the semi-cornified cell.

3) Large semi-cornified cells (Fig. 35). In their extreme form these are greatly flattened, scale-like cells in which all trace of structure has disappeared. Keratinisation/

Keratinisation never proceeds to the stage that is usual in the rat and mouse, however, for the cells remain either neutrophilic or faintly bosophilic in their reaction to the Giemsa stain: they never become eosinophile as they do in the mouse. With Delafield's haematoxylin and eosin they stain a dirty blue colour, with Heidenhain's haematoxylin jet black, and with picric acid, bright yellow. These cells have completely lost their lability: they are never found adherent in sheets and show no tendency to adhere to glass. Their outlines are sharply angular and artificial deformations of the cell body are permanent.

The description given applies to the extreme condition which is seldom attained. Inter-:mediate stages between the healthy epithelial cell and the fully cornified cell are common, however. Incipient cornification is first evidenced by the loss of lability in the cytoplasm and the disappear-:ance of vacuoles. The nucleus then begins to shrink considerably and its staining reaction becomes increasingly basophilic: finally the nucleus loses all structure and affinity for stains disappears. The nucleus usually retains its integrity throughout but in some cases the membrane ruptures. In a few cells basophilic, neutrophilic, or eosinophilic granules have been observed (Fig. 35): these may fill the/

the entire cell body. The nature and significance of these granules is uncertain but from the position of granular cells in the epithelium (see page 266) it appears probable that they represent a stage in the process of keratinisation. The granules are not products of nuclear breakdown since they are found in cells with intact nuclei and occasionally in apparently healthy epithelial cells.

4) Small completely cornified cells. These are the smallest epithelial cells found. They are irregular in outline and completely lacking in structure. They stain an intense red with both Giemsa and eosin, and jet black with Heidenhain. These cells never occurred in the experimental ewes and they were found in very few of the slaughter-house ewes. Their relation to the other cell types is uncertain. In vaginal sections they occur singly, not only in the surface layers but in the deeper epithelium, in which they lie isolated.

Cyclical changes in the representation of epithelial cell types.

The large healthy nucleated epithelial cells are the normal vaginal cells; they occur, therefore, in smears taken at all stages of the reproductive cycle, but are essentially characteristic of the resting phases of the vagina, that is, of anoestrum, dioestrum, and early oestrum. The small nucleated cell occurs only when the epithelium is/

is entirely quiescent, i.e., in anoestrum. Slight keratinisation, consisting principally of an assumption of definite form is also part of the normal life cycle of a vaginal epithelial cell, but the more heavily cornified cell in which the nucleus has commenced to contract and to lose its affinity for stains, is produced only under definite physiol-:ogical conditions. Such conditions prevail, in the sheep, during late oestrum and metoestrum. During this period there was almost invariably, in the experimental ewes, a decided tendency towards kerat-:inisation of the desquamating epithelial cells and it was during this period alone that the extreme type of cornified cell shown in Fig. 35 was encountered in the smears. The degree of keratinisation found varied considerably at successive cycles in the same ewe and from one ewe to another. Thus in some ewes the extreme type was produced at each cycle: in others keratinisation seldom went beyond a loss of lability in the cytoplasm with perhaps some nuclear shrinkage. Differences of the same nature but less in degree were found between successive cycles in the same ewe. Only rarely were keratinised cells the only type of epithelial cell present in smears, even in metoestrum: usually at least 10% of the cells were of the healthy type.

While thus essentially characteristic of metoestrum,/

metoestrum, keratinisation is by no means entirely confined to that period but occurs sporadically in all phases of the oestrous cycle, and also during the last few weeks of anoestrum, preceding the onset of the first heat period of the breeding season. It does not occur, however, except in a few odd cells, during anoestrum proper or in pregnancy. Sporadic waves of keratinisation are usually of slight intensity and the extreme type of cornified cell is not produced.

The occurrence of cornified cells in the 420 smears taken from slaughter-house material is summarised in Table VIII. From this table it may be seen that keratinisation of the vaginal epithelium was, as in the experimental ewes, essentially characteristic of metoestrum. While keratinised cells were found in a fair percentage of the smears from other stages it was rarely that more than a small percentage of the epithelial cells was affected. In metoestrum, on the other hand, cornified cells occurred in most of the smears and nearly always in high concentrations.

Keratinisation and intensive desquamation of cells from the vaginal epithelium are clearly closely correlated phenomena. But they must be regarded as parallel phenomena due to a common cause rather than as cause and effect, for in many instances heavy desquamation is unaccompanied by any trace of keratinisation/

TABLE VIII.

Keratinised Cells in Slaughter House Ewes.

	Percentage of Ewes with some keratin-:ised cells in vaginal smear.	Percentage with above 30% of epithelial cells keratinised.	Percentage with above 70% of epithelial cells keratinised.
Classes I & II (Anoestrum)	12 %	7 %	1.5%
Class III (oestrum)	24 %	24 %	8 %
" IV (early metoestrum)	38 %	37 %	25 %
" V (metoestrum)	81 %	72 %	61 %
" VI (dioestrum)	19 %	16 %	8 %
" VII (di-pro-oestrum)	35 %	5 %	0 %
" VIII (pregnancy)	0 %	0 %	0 %

keratinisation and vice versa. Keratinisation bore the same relation to sporadic desquamation taking place at times other than metoestrum that it bore to metoestrous desquamation.

ii) Lymphocytes and leucocytes.

Three types of white blood cell were found in the vaginal smears, lymphocytes, neutrophilic polymorphonuclear granular leucocytes, and eosino-:philic granular leucocytes. All three types were usually degenerate (see Fig. 36). The polymorphs had usually greatly lobed nuclei (up to 15 lobes were counted): they were apparently immobile and exhib-:ited no phagocytic behaviour in wet smears. They were very seldom found within the bodies of epithelial cells. Occasionally, however, infiltra-:tions of healthy, actively phagocytic polymorphs took place, and these were commonly found within epithelial cells.

Of the three types, neutrophilic poly-:morphs alone occurred with any regularity during the oestrous cycle. Eosinophilic granular leucocytes did not occur at all during the oestrous cycle but only during pregnancy and the puerperium, and then only rarely and in small numbers. Lymphocytes were found occasionally in the smears from both experiment-:al and slaughter house ewes but never in high concentrations: in no smear did they exceed about 5%/

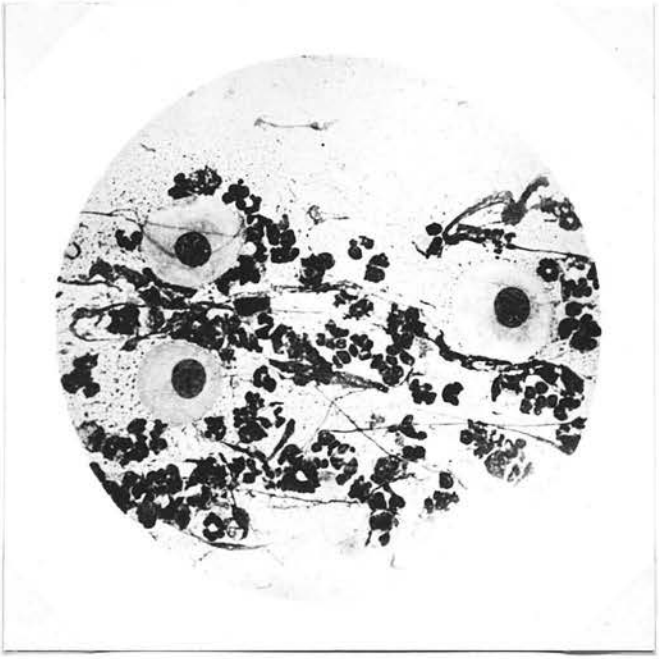


Fig. 36. Showing the degenerate type of polymorph found in vaginal smears and the peculiar "stringy" detritus to which the leucocytes give rise. x 400.

5% of all the leucocytes present. They occurred at all stages of the oestrous cycle, in anoestrum and in pregnancy: their presence could not be related in any way to the stages of the oestrous cycle but they occurred most frequently in the experimental animals after heavy infiltrations of healthy polymorphs.

Polymorphonuclear neutrophilic leucocytes, on the other hand, were found in a great many of the smears and certain cyclic variations in their number were indicated. Since smears from the three sources of material differed considerably in this respect it is intended to deal with each group separately.

In estimating the number of leucocytes present in a smear no attempt was made to obtain accurate counts: approximate estimates of the relative numbers of leucocytes and epithelial cells, based upon rough counts in a number of areas of the smear, were made and the number of leucocytes expressed as a percentage by number of the total cell content. The employment of more accurate methods than these would be futile under the circumstances, since the leucocyte content varies enormously from day to day. Nor was any allowance made in estimating these percentages for the occurrence of lymphocytes in the smears. Since lymphocytes never represented more than 5% of the leucocyte/

leucocyte content no serious error could be made by including them under the general heading of "leucocytes".

In experimental ewes.

The changes in the leucocyte percentage in a number of the experimental ewes have been represent-:ed graphically in the smear records (see Appendix). It must be remembered that these graphs represent simply the relative numbers of leucocytes and epithelial cells present in the smears: If the order of the figures in the ordinates were reversed the graphs would become "epithelial cell percentage" graphs since leucocytes and epithelial cells are the only elements involved. They would indicate changes in the actual numbers of one component only if the other remained constant, and even then imperfectly.

As may be seen from a cursory examination of the graphs the leucocyte percentage fluctuated rapidly between very wide limits: leucocytes were frequently entirely absent on one day, constituted 95% by number of all cells present twenty-four hours later, and were again entirely absent on the following day. These fluctuations occurred without any apparent relation to the stage of the oestrous cycle and they occurred also in anoestrum. Under such circumstances it is clear that any general tendency towards cyclical variation in leucocyte content in accordance/

accordance with the oestrous cycle would be complete-:ly masked by sporadic variations. For this reason and also because it was believed that, with regard to leucocytic changes, the experimental ewes did not reflect normal conditions, leucocyte counts were made for only a relatively small number (692) of the experimental ewe smears.

There appeared to be only one change in leucocyte percentage which recurred in most cycles, i.e., a depression in early metoestrum, coincident with the increase in total cell content that occurs at this time. This drop was almost certainly brought about by a great increase in the number of free epithelial cells and not by cessation of leu-:cocytic infiltration. Most of the fluctuations in leucocyte percentage occurring at other times were unaccomapnied by much change in total cell content and were probably due to irregular leucocytic in-:filtration.

Out of the total of 692 smears from experi-:mental ewes that were examined 461 (68%) contained leucocytes. The mean percentage by number of leucocytes in the whole group was 28.4%.

In non-experimental ewes.

Leucocytes occurred in only 13 out of the 156 smears taken from the breeding stock of ewes at the Institute. All the smears in which they occurred/

occurred were of the "resting" type with very low cell contents and healthy nucleated epithelial cells only. In two cases leucocytes constituted more than 90% of the cells present: the remaining 11 all showed concentrations below 10%

In slaughter house material.

The occurrence of leucocytes in the 420 smears from slaughter house material is summarised in Table IX.

The leucocyte contents were very seldom high. Out of the 132 smears in which they occurred only 22 showed concentrations above 50%. In 30 smears there were less than 5% leucocytes. Of the 22 smears with concentrations above 50% two were from ewes in Classes I and II, 4 from Class III, 2 from Class IV, 12 from Class VI, and 2 from Class VII.

The figures in the third column are an expression of a single variable, i.e., the incidence of leucocytosis. Those in column four express the same variable and, in addition, the variation in the intensity of leucocytosis, the two being summated. Thus, these figures express the chance of encounter-:ing a leucocyte as opposed to an epithelial cell in vaginal smears taken from ewes in the various phases of reproductive activity. In the last column the "incidence of leucocytosis" variable has been eliminated and the figures indicate the mean concentration/

TABLE IX.

Occurrence of Leucocytes in Slaughter House Smears.

Ovarian Class	No. of Smears	No. with Leucocytes	Percentage with Leucocytes	Mean Leucocyte Percentage. All smears	Mean Leucocyte Percentage. Those with leucocytes
I & II (Anoestrum)	114	20	17	3.0	17.1
III (Oestrum)	48	14	29	7.7	26.4
IV (early metoestrum)	42	14	33	5.0	15.0
V (metoestrum)	56	14	25	1.3	5.2
VI (dioestrum)	92	50	54	14.2	26.1
VII (di-pro-oestrum)	30	15	50	10.0	20.0
VIII (pregnancy)	38	5	13	3.0	22.8
Mean Values for all Ewes	420	132	31	6.5	20.6
Mean Values for all Ewes in Oestrous Cycle (III-VII)	268	107	40	8.4	21.1

concentration of leucocytes reached when they did occur. It is clear from the "percentage with leu-:cocytes" column that, if conditions in anoestrum and in pregnancy be taken as the standard, there is an increased tendency towards leucocytic infiltration of the vagina during the entire oestrous cycle. This tendency is particularly marked during "dioestrum" from about the 7th day of the cycle to the onset of a new heat period; that is, during the greater part of the active life of the corpus luteum and during its regression. During this period the number of ewes showing leucocytes in their vaginal smears is, on the average, doubled. The apparent slight depression in value during Stage V is probably artificial. During this period there is a great increase in the number of epithelial cells in the vagina: the vaginal smear therefore represents a much smaller proportion of the total vaginal contents than is ordinarily the case, and where only a small number of leucocytes is present the smear may contain none. The value for Stage IV may be slightly de-:pressed for the same reason. The value for Stage III is perhaps underestimated slightly, the disturbing factor in this case being the great quantity of mucus usually present in the vagina at this time. Such inaccuracies as may have been made in determining the incidence of leucocytosis in Groups/

Groups III, IV and V, could not possibly have account-:ed for the discrepancies between these groups and Groups VI and VII however.

From the last column of the table it may be seen that the concentrations of leucocytes found during the "resting" phases of the oestrous cycle are not significantly different from those found in anoestrum and pregnancy. During metoestrum there is a sharp drop in leucocyte percentage. The high-:est value encountered in Group V was 10%, but it appears to be entirely probable that this fall is brought about by the great increase in the number of epithelial cells that takes place during Stages IV to V and does not represent any decrease in the number of leucocytes traversing the vaginal epithelium. It is interesting to note that the considerable in-:crease in the percentage of the ewes showing leu-:cocytosis during Stages VI and VII was unaccompanied by any increase in the average intensity of leuco-:cytosis.

The results obtained from the analysis of post-mortem material are thus essentially in agree-:ment with those derived from the non-experimental living ewes. The occurrence of leucocytes seemed to be less frequent in the latter but this was probably due to the small number of smears examined.

It has been noted previously that the mean values/

values for "total free-cell content" of dioestrous smears were slightly higher for experimental than for control animals. This was most probably due to the greater intensity of leucocytosis in the former group.

General statements with regard to leucocytes in the vaginal smear may now be made. Leucocytes may appear in the vaginal smear of a ewe during any phase of the reproductive cycle, and, except when their proportion is reduced by intensive desquamation of the vaginal epithelium, they may constitute as much as 98% of all the cellular elements of the smear. But they occur much more frequently in smears taken during the oestrous cycle and particularly during the latter half of the cycle, than in smears from anoestrous or pregnant animals. There are clearly two alternative explanations of these relations. Ewes may differ from one another in their tendency to exhibit leucocytosis of the vagina: in some, leucocytes may penetrate at all times, in others, only during dioestrum. Alternatively, leucocytosis may occur at intervals in all ewes and at all times but with least interval between successive infiltra-:tions during the second half of the oestrous cycle. Possibly, again, both factors are involved. The data available do not provide the necessary evidence for a choice to be made between these alternatives.

In/

In the experimental ewes rapid and extensive in-:creases in leucocyte percentage were the rule rather than progressive increments: Leucocytes appeared to be incapable of living any length of time in the environment of the vagina and soon disappeared after an infiltration. It might be concluded therefore that the behaviour of leucocytes in the normal animal is similar to that in the experimental ewes and that the increased incidence of leucocytosis in the slaughter-house material during the oestrous cycle was due to more frequent occurrence of leucocytic infiltration during that period. But, on the other hand, it will be shown in dealing with the histology of the vagina that the occurrence of leucocytes in slaughter-house smears was due to strictly localised infiltrations of polymorphs, these being entirely absent from most of the mucosa. This circumstance favours the view that most ewes never show leucocy-:tosis at all, and possibly a greater number show it in dioestrum.

Mechanical stimulation of the vaginal mucosa such as that involved in taking smears increases the tendency towards leucocytic invasion considerably. It will be recalled that leucocytes occurred in 68% of the experimental ewe smears as compared with 31% in the most-mortem material and 9% in the non-experimental living material.

The data given by Cole and Miller (40, 42) and/

and by Darlow and Hawkins (47) with regard to the occurrence of leucocytes in vaginal smears indicate that conditions in their animals were similar to those in the experimental animals used by the present writer. The "cyclical changes" described by these investigators were probably largely the effects of the artificial conditions induced by smearing and the discrepancies between the accounts given may be ascribed to the same cause.

The increased polymorph infiltration was the only difference established between vaginal changes in experimental and control animals apart from the more frequent occurrence of vaginal bacteria in the former.

E. Changes in the Vaginal Contents during Anoestrum and immediately prior to the Commencement of the Breeding Season.

During the summer months, when the ovaries are completely quiescent, the vaginal smear is monotonously similar from day to day. The type of smear found is the same as that usually found in di-:oestrum, i.e., it consists of a few cells suspended in a variable, but always small, amount of rather "stringy" mucus. The cells present are usually all epithelial cells, mainly healthy but occasionally slightly keratinised. A few leucocytes may be present./

present. At about mid-September, that is about six weeks before the commencement of the breeding season, the smear begins to lose its monotonous character and considerable but irregular changes commence to take place, both in the mucus and in the cell content. (See graphical records in Appendix.) At first there is no suggestion of regularity in these changes but during the 2 to 4 weeks preceding the first heat period the changes become periodic and are clearly comparable to those which take place during the oestrous cycle. This is true especially of the changes in total cell content. In practically every case the total cell curve shows a marked rise about 14 days prior to the first heat: after this peak the irregular changes virtually cease. A similar change sometimes takes place in the mucus graph but in this case the alteration is not so marked nor so regular in its appearance. In some cases there are indications of two such cycles preceding the first oestrous period. Changes in the microscopical character of the smear also take place, the epithelial cells composing the large desquamations frequently being keratinised.

It has been noted that one or more crops of follicles rupture and develop into corpora lutea before the first heat period has occurred. It appears to be indubitable therefore, that the cyclical changes in the vagina occurring prior to the/

the commencement of the breeding season are accompanied by, and bear the same relation to ovulation that is the case with the later cyclical changes in association with which heat is expressed. In ocrroboration of this view it may be noted that at the unilateral ovariotomy operation performed on ewe 705 a corpus luteum approximately $3\frac{1}{2}$ days old was found in the ovary: from the graphs it may be seen that marked peaks in the mucus and cell graphs occurred four days and one day respectively prior to the operation, i.e., at about the time the follicle ruptured in the ovary. Also, the five ewes killed at the Institute during the last few weeks of anoestrum and showing ovarian activity all had the types of vaginal smear characteristic of the stages of ovarian activity at which they were killed, although none of them had come into heat.

After the last heat period of the breeding season the vagina usually passes at once into a state of quiescence. In one case, however (E.E. 303), a typical vaginal cycle was passed through 16 days after the last heat period without the expression of heat. Cole and Miller (40) state that the smear does not assume a monotonous character until some time after the last heat period.

F. <u>The Diagnostic Value of the Vaginal Smear</u>.

In/

In the mouse, rat, guinea-pig, etc., the changes in the vaginal smear are of a sufficiently definite and characteristic nature to allow them to be used as reliable diagnostic measures in the determination of phases of ovarian activity. In the sheep there are three alterations in the smear which might be utilised in the same manner, (a) the flow of mucus with low cell content, characteristic of oestrum, (b) the sharp rise in total cell content of the vagina giving rise to the abundant, dry, "cheesy" smear, characteristic of early metoestrum, (c) keratinisation of the epithelial cells coincident with the rise in total cell content. Each of these is very characteristic of its particular phase but none of them is of absolute diagnostic value, positive or negative. They all occur sporadically at times other than those of which they are essentially characteristic and they are frequently only slightly expressed during the phases of which they are typical. Smears taken over a period of days may be relied upon to give an accurate indication of ovarian changes but the finding of a "type" smear at a single examination or at autopsy does not justify any definite conclusion as to the functional state of the ovaries. The occurrence or number of leucocytes in a smear is no diagnostic indication. The macroscopical changes in the vaginal/

vaginal sample are more consistently expressed than the microscopical, so that, for purely diagnostic measures, the preparation of stained smears is not justifiable. The present author is unable to agree with the conclusion of Cole and Miller (42) that it is possible to distinguish between pro-oestrum and oestrum on the basis of the time of commencement of the mucus flow of oestrum.

4. HISTOLOGICAL CHANGES IN THE VAGINAL MUCOSA.

Casida and McKenzie (37) have described in detail the histology of one area of the vaginal mucosa in nine ewes autopsied at different intervals after the commencement of oestrum, and have defined a number of cyclical changes occurring during the dioestrous cycle. All the histological appearances described by these authors have been observed during the present investigation, but in no single instance have the relations of these changes to the oestrous cycle been confirmed and in some cases the findings are contradictory. These discrepancies are believed to be due to the great amount of regional and individual variation which exists, and to which Casida and McKenzie were unable to give adequate consideration on account of their scanty material. Regional variation, except during anoestrum, is enormous,/

enormous, and it is not uncommon to find the two extreme types of mucosa in different parts of the same vagina. It is rarely that the character of the epithelium is uniform over the whole length of a centimetre long section of vagina, and the variation may be considerable.

A. The Stroma.

The only cyclical changes observed in the sub-epithelial parts of the vaginal wall were those in vascularity and oedema, the relation of which to the oestrous cycle have already been indicated (page 208). Histologically, these changes take the form of increased blood content of the capillaries, and increases in the number and size of lymph spaces in the connective tissue during oestrum. Haemorrhage has not been observed.

No structural alterations were observed in the smooth muscle cells but these were not subjected to detailed cytological examination.

B. The Thickness of the Epithelium, Epithelial Papillae and Folding of the Mucosa.

The thickness of the vaginal epithelium is, on the whole, greater during the oestrous cycle than at any other time. In anoestrum it is at its lowest level, when an average of about 4 layers is usual./

usual. This is only slightly thicker than in the prepubertal animal. During the oestrous cycle the average number of cell layers is usually about 6. The greatest average thickness is reached during late oestrum and early metoestrum (Stages III and IV) just prior to the desquamation of the surface layers, when 10 to 12 cell layers are not uncommon. The lowest level is attained during late metoestrum (Stage VI) immediately after the dehiscence. From this time the thickness increases gradually up to the time of the next dehiscence, or, if the ewe has been served and conceived, until about the 3rd week of pregnancy. The local club-like thickenings of the epithelium are also more frequent and more strongly developed during the oestrous cycle than at other times: they do not, however, show definite variation during the oestrous cycle. Folding of the mucosal surface is not more frequent during any one stage of the re-:productive cycle.

C. Frequency of Mitosis.

Mitoses are usually confined to the basal layer or stratum germinativum of the epithelium but occur rarely in the second layer. Mitosis is never intense; the greatest number of typical mitotic figures observed at any time was 19 per centimetre section/

section (6 μ thick). It occurs more frequently during the oestrous cycle than at other times: in anoestrum, mitoses were rare and in many sections were entirely absent. There is no definite peak of mitotic activity during any one stage of the oestrous cycle. Cell divisions were, on the average, slightly more numerous in Stage III (early oestrum) material than in other stages but this may have been due to the comparitively small number (34) of Stage III vaginae examined. There was certainly no increase during metoestrum and dioestrum such as was found by Casida and McKenzie. All the mitoses in any one section are commonly at the same stage. This suggests that cells divide synchronously in any given area.

D. The Morphology of the Superficial Layers of the Epithelium.

Four main types of cell were encountered in the superficial layers of the vaginal epithelium.
1) Large, rather flattened cells varying in form from fusiform to cubical with oval or spherical nuclei, shrunken but still reticulate in structure (Figs. 32 and 37). The cytoplasm stains faintly with eosin and faint grey with iron haematoxylin. Cell boundaries are usually rather poorly defined but may be very distinct. The cytoplasm is frequently vacuolated/

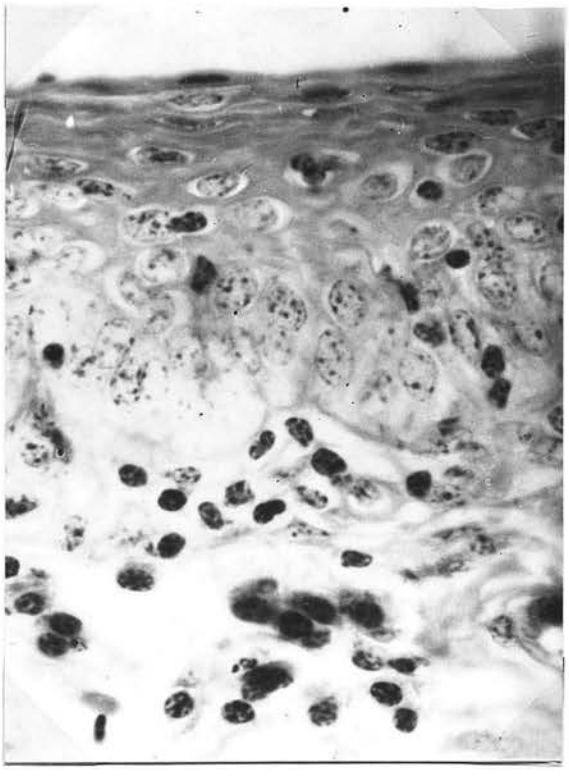


Fig. 37. Normal "resting" vaginal mucosa. The superficial cells are nucleated and not keratinised. Lymphocytes are concentrated beneath the epithelium and invade the latter. Ewe killed in dioestrum. x 800.

Identical with Fig. 30

vacuolated. This type of cell corresponds to the "large healthy nucleated cell" of the vaginal smear.
2) Greatly flattened, scale-like cells with a shrunken, dense nuclei, or sometimes no nuclei at all (Figs. 38 and 40). The cytoplasm usually stains more intensely with eosin than in the above type. Their reaction to iron haematoxylin varies considerably but they usually stain jet black, indicating keratinisa-:tion (Fig. 39). This type of cell, in its more extreme form, corresponds to the "semi-cornified cell" of the vaginal smear.
3) Large polygonal cells with spherical, but very faintly staining nuclei and very clear cytoplasm not staining with either eosin or iron haematoxylin (Fig. 41).
4) Large cuboidal or columnar cells with healthy, spherical or elongated nuclei and very abundant cytoplasm. (Fig. 42) This type of cell seems to be homologous with the "mucus" cell found in the vaginae of the mouse and rat during pregnancy.

The last two types of cell have not been recognised in vaginal smears. The cytoplasm of the last type is fully labile and, in smears, they probably flatten out and become indistinguishable from the first type.

The first type of cell may be described as the normal surface cell: it is the type characteristic/

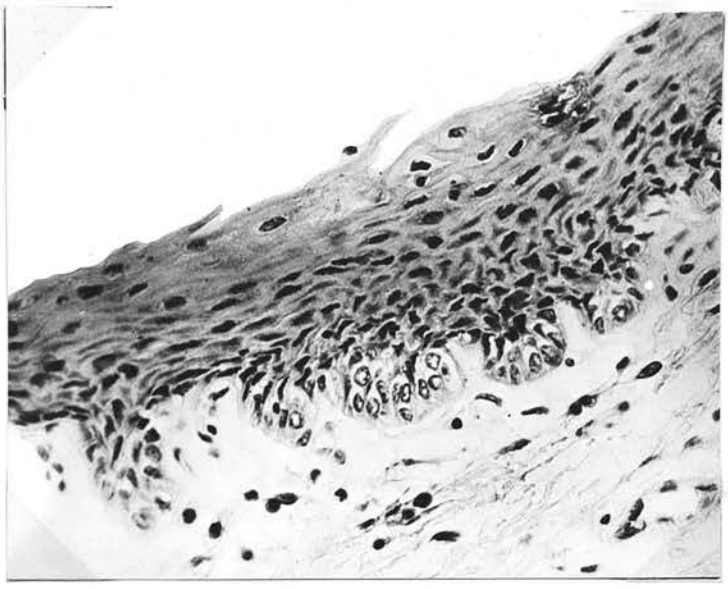


Fig. 38. Keratinised vaginal epithelium of the extreme type. Nuclei are greatly shrunken and are absent from some cells. Only the papillae remains unkeratinised. Stained with Delafield's haematoxylin and Eosin. Ewe killed in late oestrum. x 400.

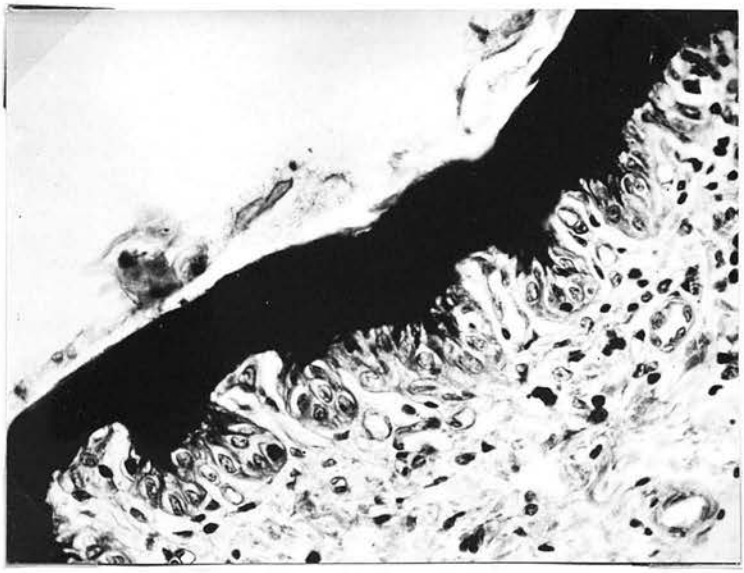


Fig. 39. Section from the same block stained with Heidenhain's haematoxylin, show-:ing keratinisation of the superficial cells. x 400.

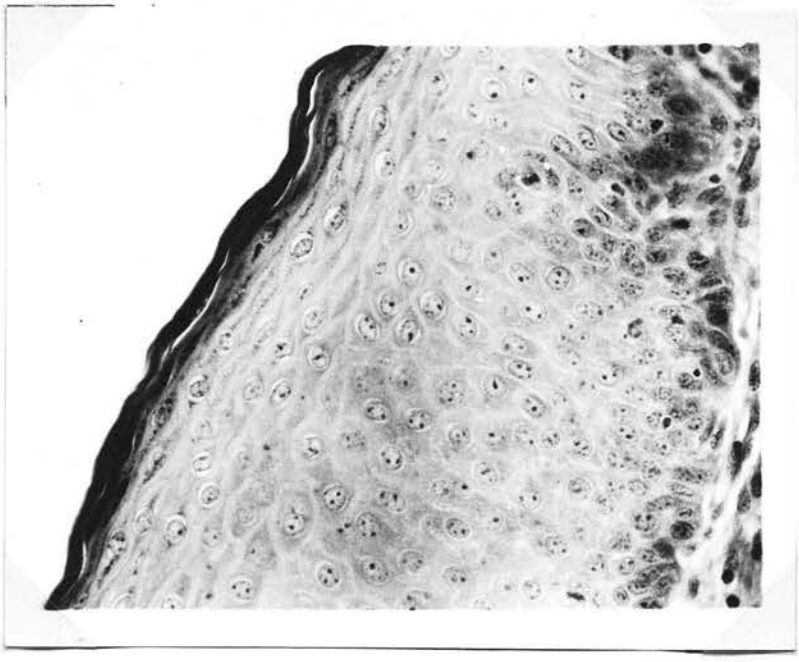


Fig. 40. Weakly keratinised vaginal epithelium showing stratum granulosum. Stained with Heidenhain's haematoxylin and differentiated in picric acid. Ewe killed in early metoestrum. x 380.

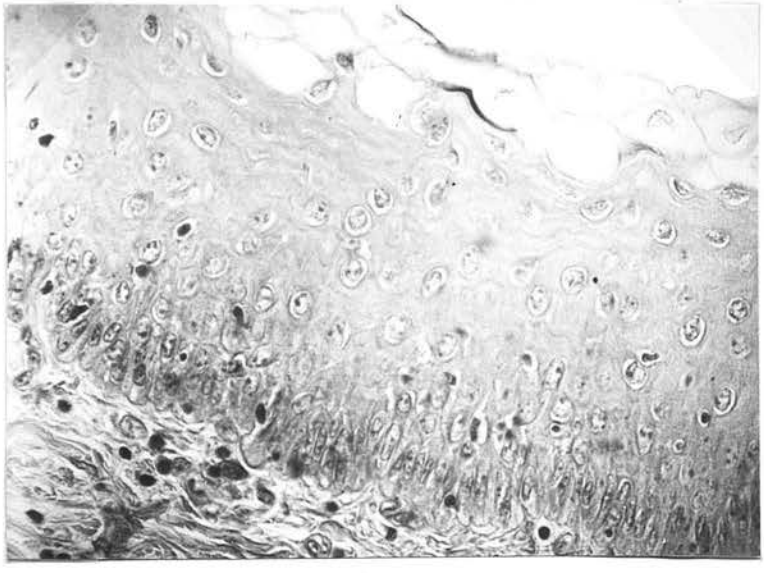


Fig. 41. Showing the large clear type of superficial epithelial cell. Stained with Heidenhain's haematoxylin. x 400.

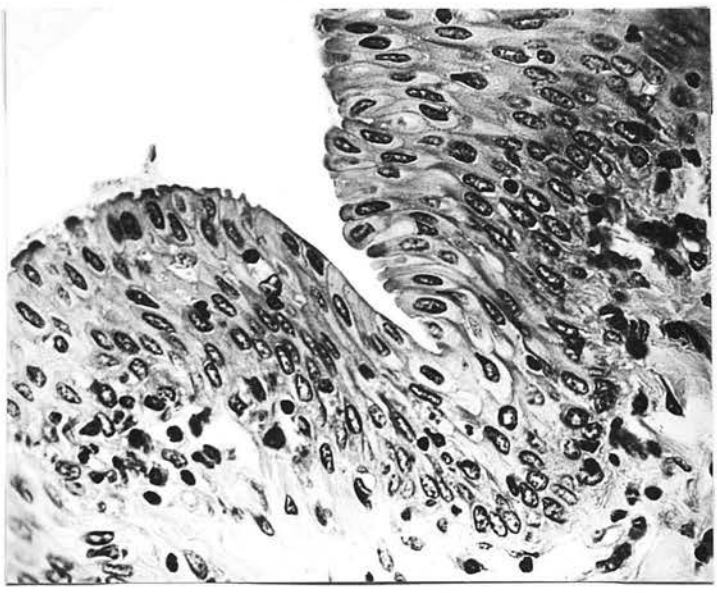


Fig. 42. Showing the "secretory" modification of the superficial layers of the vaginal epithelium, typical of pregnancy and sometimes found in late dioestrum. Staining with Delafield's haematoxylin and eosin. x 450.

characteristic of anoestrum and of the immature animal and is the only type that is ever found continuously over the whole epithelium.

The tendency towards excessive flattening and the formation of the second type of cell is greatest during oestrum and metoestrum (Stages III and IV). They are by no means confined to these periods, however; patches of them are to be found in most vaginae irrespective of the stage of reproductive activity. These cells are never continuous over the whole vaginal surface even during Stage IV but occur in patches, seldom more than a centimetre wide. As a rule, not more than 3 or 4 layers of cells are thus flattened but occasionally the entire epithelium, including the stratum germinativum consists of these cells, only the bases of the papillae being unaffected (Fig. 39). Quite commonly an intra-epithelial zone of flattened cells is found, the actual surface being composed of degenerate type one cells.

The third type of cell was found in three anoestrous and four oestrous ewes. The origin and nature of this type have not been elucidated.

A columnar "secretory" surface layer is essentially characteristic of the later stages of pregnancy (see page313). Occasionally, however, patches of this type of cell are found during the last few/

few days of the oestrous cycle (Stage VII), especial-:ly near the cervix.

E. Keratinisation.

The extremely flattened "type two" cells are usually keratinised. Keratinised cells may thus appear in the superficial epithelium at all stages but particularly in late oestrum and early met-:oestrum. The less markedly flattened cells are not as a rule keratinised, but when a non-acid fixative is used and sections stained with iron haematoxylin, some trace of keratinisation may usually be detected. This is frequently the case also with the polygonal cells of the intermediate layers. Where a fully keratinised superficial layer is present there is usually a layer of coarsely granular cells immediate-:ly beneath this (Fig. 40). These granular cells clearly constitute a stratum granulosum homologous to that of the epidermis and they are probably an intermediate stage in the process of keratinisation.

The most prominent feature of the keratini-:sation phenomenon in the sheep is its close locali-:sation. In some cases the entire epithelium in-:cluding the stratum germinativum may be strongly keratinised in one area while in an adjacent area no trace of keratinisation is shown.

The great local variation in the occurrence of/

of keratinisation makes any attempt to analyse its relation to the oestrous cycle from examinations of vaginal sections very difficult. The vaginal smear gives a fair representation of the character of the surface epithelium as a whole and is therefore a much more reliable criterion for this purpose. An analysis of the occurrence of keratinised cells in the vaginal smear has already been given (pages 238-241).

F. Autolytic Degeneration of the Superficial Layers of the Epithelium.

Autolytic degeneration of the surface layers of the epithelium is constantly taking place. It does not greatly affect keratinised cells, however. Degeneration is most pronounced when the epithelium is thickest and is usually absent in very thin epithelia. When polymorphonuclear leucocytes are concentrated in the superficial layers of the epithelium degeneration is more pronounced. Autolysis of the surface epithelium is responsible for the appearance in the vaginal smear of isolated nuclei of epithelial cells. The free surface of the cell becomes autolysed, setting free the nucleus while the remainder of the cell body remains attached to the epithelium.

G./

G. Desquamation.

The casting off of the superficial layers of the vaginal epithelium is directly responsible for the sharp rise in the total free-cell content of the vagina that takes place in metoestrum. The chronol-:ogical relationship of this process to the oestrous cycle need not be discussed again. Slight desquamation takes place continuously from the epithelium but only in the form of odd cells here and there. It is more pronounced in thick epithelia than in thin. At the metoestrous desquamation about four or five layers are cast off simultaneously from practically the whole vaginal epithelium. The dehiscence is most marked near the cervical end of the vagina. Where keratinisation has affected the stratum germinativum the entire epithelium is cast off and the epithelium is replaced, presumably, from the surrounding areas unaffected by keratinisation, and from the epithelial papillae, which also escape the process. Keratinisation can in no sense be regarded as the cause of desquamation, however.

There is no desquamation from epithelia covered by the columnar "secretory" type of cell.

H. Lymphocytes.

Lymphocytes are constantly present throughout/

throughout the superficial connective tissue and the base of the epithelium of the vagina. Their number is greater, however, during the oestrous cycle and pregnancy than during anoestrum. Their number was greater also in the experimental ewes than in control animals. They are not subject to cyclical alterations in numbers or distribution. They are much more numerous in the vestibule, where there are many lymph nodes, than in the vagina.

J. Neutrophilic Polymorphonuclear Leucocytes.

These were encountered in too few of the non-experimental ewes to allow any conclusion to be drawn as to their relative frequency in different stages. It has been shown previously that they occur more frequently in the vaginal lumen during dioestrum than at other times (page 250). Leucocytic infiltration of the vagina must be a very localised phenomenon for polymorphs were found in the vaginal sections from only a few of the animals in which they occurred in the vaginal smear, and they do not originate from the higher parts of the reproductive tract.

K. Eosinophilic Leucocytes.

These occurred in the superficial connective tissue/

tissue and the epithelium of five vaginae, all of which showed the columnar "secretory" type of cell in the superficial epithelium.

L. The Glandulae vestibulares majores.

No variation in the structure of Bartholin's glands was observed but the glands were encountered in only nine animals.

X : CYCLICAL CHANGES IN THE CERVICAL CANAL.

1. THE ANATOMY AND HISTOLOGY OF THE CERVIX.

The cervix, in the sheep, is a passage some two inches in length. The mucosa is thrown into a great number of deep longitudinal folds (see Fig. 57). The whole wall, also, particularly near the external os, is thrown up into stout annular or tongue-like folds projecting towards the external os (see Fig. 25). These, under normal conditions, interleave with one another so that the lumen is almost completely occluded in the lower parts of the cervix and it is quite impossible to introduce any instrument into the uterus by way of the cervical canal. One or more of these folds constitutes the external os. At the internal os the folded mucosa of the cervix is replaced abruptly by a mucosa resembling that of the uterus except for the very small size and attenuated form of the cotyledons. A comparative account of the anatomy of the cervix in the domestic animals has been given by Trautmann (160).

The characteristic epithelium of the cervix is made up of tall columnar, mucus-secreting cells (Fig. 43). These are usually very narrow and the oval nuclei are confined to the basal half of the cell. The free ends of the cells are not as a rule,/

rule, attached to one another. At the internal os this epithelium grades quite insensibly into the non mucus-secretory columnar epithelium of the uterus. Near the external os the columnar epithel-:ium is replaced by the stratified squamous epithel-:ium of the vagina: there is no gradual transition from one to the other but the two commonly overlap, so that the stratified epithelium is covered by a superficial layer of columnar mucus-secretory cells (Fig. 46).

The epithelium is demarcated from the sub-epithelial mucosa by a well-defined basement membrane. The stroma forms only a very thin layer seldom more than half a millimetre thick at the bases of the mucosal folds. The connective tissue fibres compos-:ing the stroma are for the most part collagenous but a few yellow elastic fibrils are sometimes present. The number of stroma nuclei increases progressively from the external to the internal os, so that there is a gradual transition from the vagina, in which there are very few stroma nuclei, to the uterus in which there are many. In the immature animal the stroma is rather dense but in the ewe there are commonly a great many small lymph spaces. The stroma is fairly vascular and frequently contains small venous sinuses. A few lymphocytes are commonly present, concentrated beneath the epithelium, and/

and only very rarely penetrating between the epithelial cells. Polymorphonuclear leucocytes have not been observed except in some of the animals from which vaginal smears were taken regularly, and in these, near the external os only.

The cervix is remarkable for the great thickness and density of the sub-mucosal muscular layers. In a mature ewe which has raised lambs there is a dense layer up to 7 or 8 mm. thick compos-:ed of smooth muscle, yellow elastic fibrous tissue and collagenous fibrous tissue. The fibres are arranged in bundles, irregularly placed, but the muscle may be roughly divided into an internal circular and an external mixed longitudinal and radial coat. The blood vessels in this dense layer are small and few in number: there are no lymph spaces of any size.

External to this there is a layer about 5 mm. thick composed of very loose connective tissue in which are contained a great many large blood ves-:sels. This is followed by a thin longitudinal coat of smooth muscle and finally by the peritoneum. The vascular coat, the external longitudinal muscular coat, and the peritoneum are continuous with the corresponding coats of the uterus and are almost identical with these. The blood supplies of the two organs are derived from a common source, the ovarian arteries.

2./

2. GROSS CHANGES.

During the greater part of the dioestrous cycle the cervix is tightly contracted, the circular folds interleaving and almost completely occluding the lumen. During oestrum, however, the muscles relax somewhat, the lumen becomes more open and it is sometimes possible to pass a flexible instrument into the uterus by way of the cervical canal. It requires considerable force to inject fluid into the uterus through the cervix of a ewe killed during dioestrum but this may be done readily in a ewe killed in oestrum. There is no evidence that the cervix or uterus undergo any co-ordinated movements, such as Hartman and Ball (69) found to exist in the rat, serving to suck the sperm into the uterus during coitus: in ewes killed a short time after normal coitus the bulk of the sperm was still in the vagina, a few sperm had penetrated to the cervix but none to the uterus.

The large blood vessels show an increased blood content in oestrum, in common with those of the vagina and uterus and there is slight congestion of the mucosa at this time.

3. HISTOLOGICAL CHANGES.

The histological changes concern only the mucosa/

mucosa and the contents of the lumen, no changes having been observed in the muscular or vascular layers, apart from the increased vascularity in oestrum.

During oestrum there is some increase in the vascularity and in the oedema of the stroma: haemorrhage does not take place. There are no cyclical changes in the number of lymphocytes in the superficial stroma.

In the distal part of the cervix, near the external os, where the epithelium is of the vaginal type, the cyclical changes are similar to those in the vagina, i.e.,the principal alteration is a considerable desquamation in early metoestrum. The desquamation is, if anything, more intense than in the vagina. Keratinisation of the superficial cells does not take place, however.

In the immature lamb mucus discharge does not occur, although a very small amount may be formed in the epithelial cells, particularly those at the bases of the deep longitudinal folds. The epithelial cells are of a very uniform, low columnar or cubical type, with round or oval nuclei. In anoestrum, conditions are similar but there is usually a some-:what greater amount of mucus formed in the epithel-:ial cells although there is rarely any trace of secretory activity and the free ends of the cells are intact./

intact. Accordingly, there is seldom any mucus in the lumen of the cervix. Throughout the dioestrous cycle the formation of mucus is greatly increased above the anoestrous level: practically all the cells, with the exception perhaps of some near the external os, commence to form mucus and most of them begin to discharge this into the lumen of the cervix. There are always patches of cells which are not discharging, however, and these become distended with mucus, the nucleus is pushed to the base of the cell and sometimes flattened out transversely (see Figs. 43 and 44). Such cells stain deep red with the mucicarmine stain. The mucus is always collected near the free end of the cell and never surrounds the nucleus. The epithelial cells of the cervix have thus the characters of the "goblet" cells of the intestinal and other mucosae rather than those of the cells of the mucus glands.

The discharge of mucus from the cervical epithelium is continuous throughout the oestrous cycle, but during metoestrum and dioestrum the secretion is tenacious and "stringy"; the mucus in the lumen remains continuous with that which is left in the cells (Fig. 45). Mucus thus collects in the cervical canal. During oestrum, on the other hand, the mucus in the cervix liquefies and flows into the vagina: the mucus which is being discharged from the/

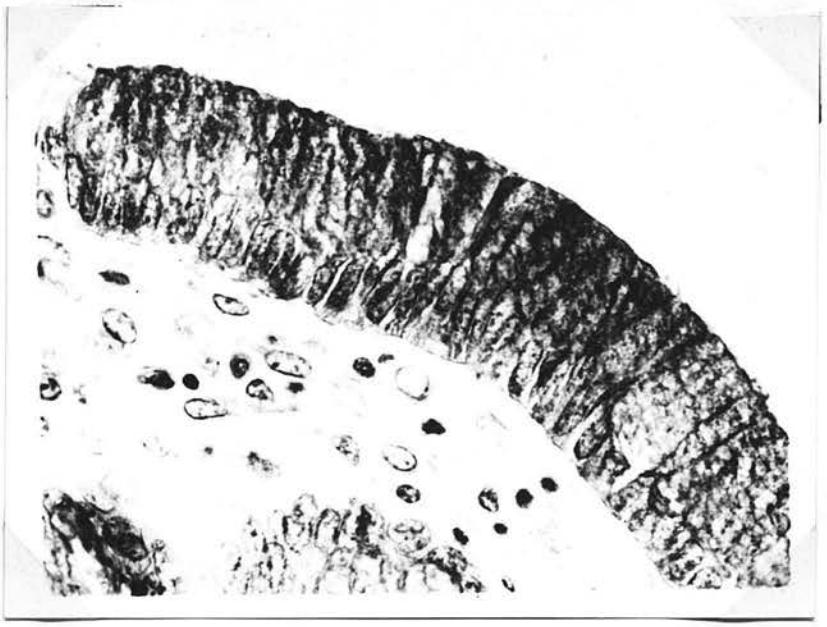


Fig. 43. Typical cervical epithelium. Stained with Delafield's haematoxylin and Mayer's mucicarmine. x 650.

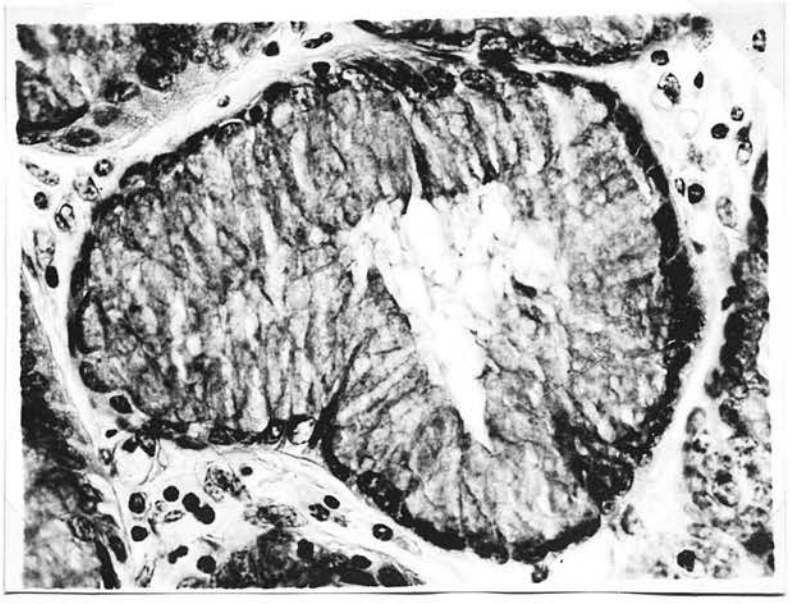


Fig. 44. Cervical epithelium choked with mucus secretion. Note nuclei pushed to bases of the cells flattened transversely. Stained with Delafield's haematoxylin and mucicarmine. x 500.

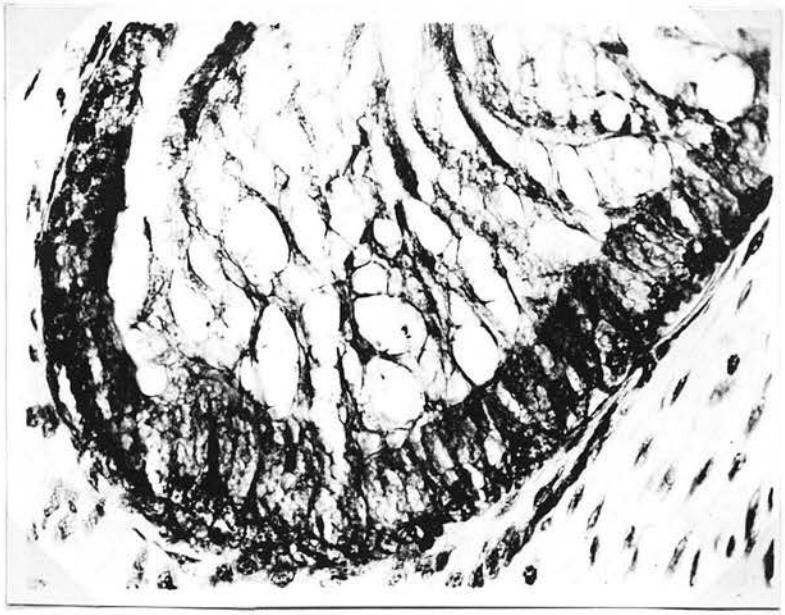


Fig. 45. Secretory cervical mucosa. Showing "stringy" mucus remaining attached to mucus still within the epithelial cells. Ewe killed in metoestrum. Stained with Delafield's haematoxylin and mucicarmine. x 400.

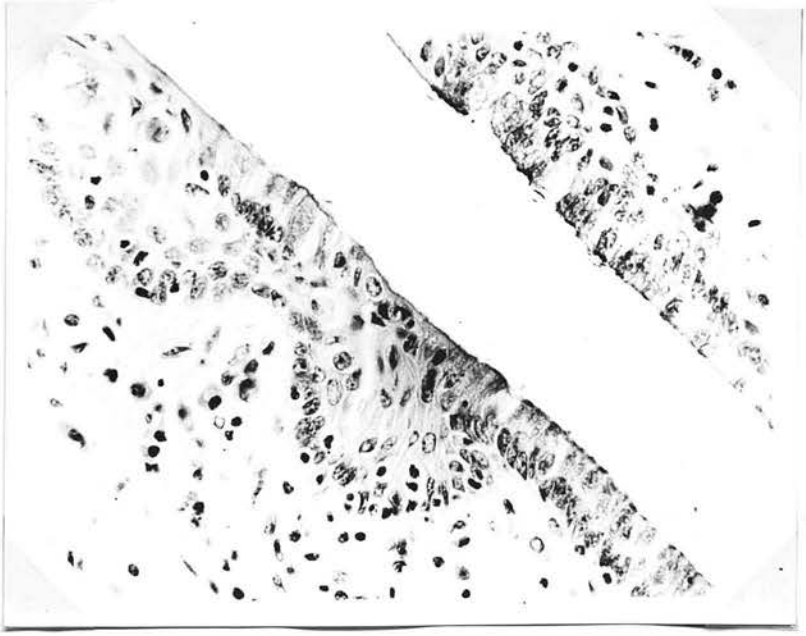


Fig. 46. Showing overlap between cervical and vaginal mucosae, with stratified epithel-:ium surmounted by columnar mucus-secretory cells. Stained with Delafield's haematoxylin and mucicarmine. x 400.

the epithelial cells also liquefies so that there are no longer strings of mucus attached to these cells. It has not been possible to determine from histolog-:ical study whether the actual secretion of mucus is increased during oestrum, but since the amount of mucus poured into the vagina at this time is many times the volume that could be held by the cervix, it may be concluded that there is a considerable increase in secretion - even when allowance is made for the swelling of the mucus during lique-:faction and for any considerable dilution by serous exhudates which may take place. Nor has histological evidence of the manner in which the liquefaction of the cervical mucus is brought about been obtained, apart from indications that the liquefaction commences near the epithelium. Presumably, since mucus is precipitated by weak acid, and dissolves in weak alkali, there is an increase in pH during oestrum: the liquefaction might, however, be brought about by enzymic lysis. Since the mucosa is sometimes congested and oedematous during oestrum there may also be a transudation of lymph or serum serving to dilute the mucus. Some fluid might also reach the cervix from the uterus, for although the uterus is usually pendant in the abdominal cavity and therefore below the level of the cervix, peristalsis is constantly taking place and/

and would carry any fluid which might be present towards the cervix. However, there is no evidence that fluid is secreted by the uterus during the dioestrous cycle.

XI : THE MAMMARY GLANDS DURING THE DIOESTROUS CYCLE.

Observations of the size of the udder and nipples, of the "feel" of the glands, i.e., whether they were turgid or flaccid, and of the amount and nature of the fluid that could be expressed from the nipples, were made on the group of experimental ewes throughout the period over which vaginal smears were taken. No cyclical variations in any of the above characters were observed. The mammary glands are considerably larger in a ewe which has borne a lamb than in an immature ewe and a small amount of milky fluid may sometimes be obtained from such a ewe.

It was not anticipated that any changes would be found to occur in the udder during the dioestrous cycle, for it is not until pregnancy is well advanced that any considerable increase in size occurs.

The mammary glands have not yet been examined microscopically.

IV : P R E G N A N C Y .

No attempt has been made to carry out a full, detailed investigation of the phenomena attend-:ant upon pregnancy. Attention has been directed chiefly to structural alterations in the uterus taking place prior to, and during the process of attachment of the foetal membranes, to the function of the uterine glands, and to the modifications of cervix and vagina during pregnancy. The main pur-:pose in undertaking these studies has been to enable parallels to be drawn, and correlations made, between the phenomena of pregnancy and those of the dioestrous cycle, so that the latter phenomena may be given a functional interpretation.

Pregnancy lasts for about 21 weeks. The lamb is born in an advanced state of development: the skeleton is very largely ossified, the coat is well developed and the eyes are open. The lamb gains the use of its limbs and is able to follow its mother a few hours after birth.

I : THE EARLY DEVELOPMENT OF THE FOETUS.

As will be seen from Appendix Table I, a number of the non-experimental ewes slaughtered at the Institute were killed at known intervals after fertile service. Foeti of known age were also obtained by caeserian section from two other ewes.

The early development of the foetus appears to be very slow. Allen et al (2) recover-:ed 4 cell stages from the Fallopian tubes 53 hours after service and 4 to 6 cell stages at 66 and 98 hours. It seems therefore that the first three segmentation cleavages occupy about 4 days. In this connection it is interesting to note that Sakurai (140) has shown that the early development of the roe-deer foetus is extremely slow, 4 to 5 months being occupied by the phase of development which is passed through in the sheep during, at most, the first 14 days. Bonnet (reproduced by Zietschmann (169) p. 145) has figured a blastocyst of the sheep recovered 12 days after coitus. This was about 240 mm. long and 1 mm. in diameter. The embryo itself was still in the form of an "embryonic shield". The earliest embryo recovered by the present author was from a ewe killed 14 days after coitus and this blastocyst was considerably smaller than that figured by Bonnet. It was about 30 mm. long and

0.5/

0.5 mm. in diameter, and there was an enlargement at each end of the tubular blastocyst. The embryonic shield was a minute accumulation of cells in which no differentiation could be detected on examination by histological sections.

Two foeti recovered from a ewe killed on the 18th day after insemination were in a stage of development slightly earlier than that figured by Bonnet (reproduced in (169) p. 146) as a foetus 17 days, 22 hours old (see Fig. 47). The amnion was complete and the foetus thus floated freely within the chorion. The chorion (i.e., combined chorion and trophoblast) of each foetus extended nearly the whole length of the uterine horn. The tip of the cyst was grotesquely branched. The cavity enclosed by the chorion contained a small amount of fluid but not enough to cause distension of the chorionic membrane or of the uterus. The allantois, still a very rudimentary organ, was distended with fluid.

The foetus itself was 3.5 mm. long. The heart was developed and there were about 15 somites recognisable. The details of structure have not yet been worked out.

A 20-day foetus is illustrated in Figs. 48 and 49. At this age the allantois, now 9 cm. long and 1.3 cm. in diameter, is turgid with fluid and causes appreciable distension of the uterus. It is/

Fig. 47. Foetus 18 days old removed from the chorion. The T-shaped structure at the tail end is the allantois and the large structure attached to the abdomen is the yolk-sac. The process above the head is part of the amniotic membrane and marks the point where the two amniotic folds fused, separating amnion from chorion. Scale graduated in millimetres.

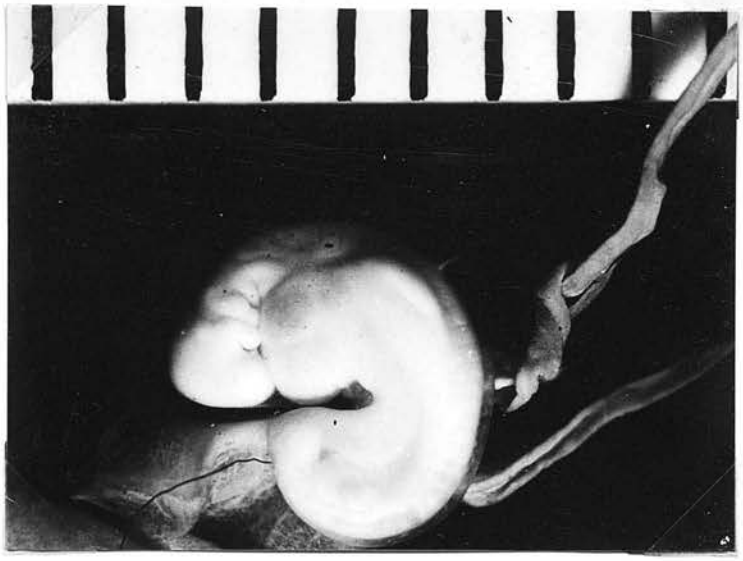


Fig. 48. Foetus 20 days old.

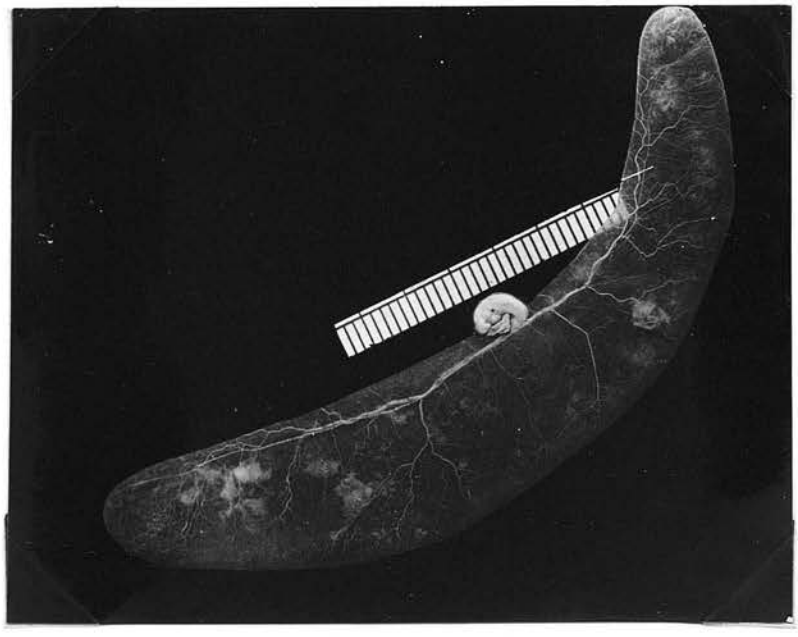


Fig. 49. The same foetus, showing develop-:ment of the allantois. The chorion has been removed, and the yolk sac severed. Note the extensive vascularisation of the allantois.

is richly vascular, a main artery and vein extending anteriorly and posteriorly almost to the tips of the allantois, and giving off a great many branches. The vessels run, naturally, on the external surface of the vesicle. The foetus and the membranes attach-:ed to it still remain completely unattached to the chorion, which is an extremely friable membrane closely adherent to the surface of the uterine mucosa. The extra-embryonic coelom contains practically no fluid. The yolk sac and amniotic cavity similarly contain no fluid.

The foeti were 5.6 mm. long. The main organs were differentiated and the body wall closed except for a small area between the yolk sac and the allantoic stalk. The brain vesicles were clearly differentiated and the eyes had commenced to form. Four gill slits were present and the first gill arch was commencing to bend forward into the position of the mandible. The anterior limb buds were well developed and the posterior were indicated.

It is of great interest to note that an abundance of red blood is present in the foetus at this age although the foetus is as yet completely unattached to the uterine mucosa and is not even in contact with the mucosa except through the chorion, with which the foetus itself has no material connection at this stage. Haemorrhage from the uterine/

uterine mucosa does not commence until about the 20th day after coitus and in this particular ewe no trace of haemorrhage was found. On the other hand it appears to be unreasonable to suppose that the egg contains sufficient iron to enable the foetus to form the quantity of haemoglobin that must be pres-:ent in the blood at this early stage.

By the twenty-second day the allantois has extended throughout the length of the uterine horn, has grown up round the foetus and completely enclosed this and has completely filled the extra-embryonic coelom. Distension of the uterus has now become quite marked. The allantois is still unattached organically to the chorion although it is now brought into close contact with this by the pressure of the fluid within its cavity. The 22-day foetus was found to be 7.9 mm. long (crown to rump length). The flexures of the body were well defined, the limb buds well defined, and the eye appeared to be fully formed. The first gill arch formed a distinct mandible.

The first evidence of attachment of the foetal membranes to the cotyledons was found in the ewe killed on the 27th day after service. The foeti were at this age 16 mm. long (compare Fig. 50). The fluid in the allantoic cavity caused considerable distension of the uterus, and there was also a considerable/

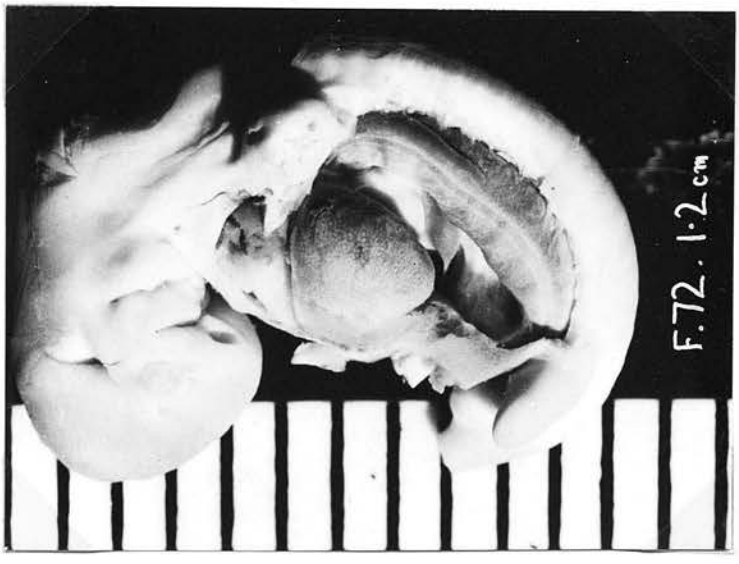


Fig. 50. Foetus about 25 days old, removed from the amnion and dissected to show internal organs. The intestine has been removed to show the development of the gonad.

considerable volume of amniotic fluid. The allantoic membrane was definitely attached to the chorion and the combined membrane was strongly vascularised. Over each cotyledon the foetal trophoblast appeared to be slightly roughened and these areas were more richly vascular than the rest of the membrane. The cotyledons themselves were flattened and were also roughened. It is thus clear that there was slight interdigitation of the foetal and maternal tissues.

At the 36th day the foeti were 27 mm. long. The foetal villi were well embedded in the cotyledons so that gentle traction was required to separate the membranes.

To summarise, therefore, the foetus is in contact with only a very small area of the uterine mucosa up to about the 15th to 16th day. Thereafter, very rapid development commences to take place both in the foetus itself and in its membranes. The chorion fills the uterus by the 18th to 19th day. Organic connection between the foetus and the chorion, which is the only part of the foetus in contact with the uterine mucosa, is completely severed from about the 16th to the 26th day. It is restored when the allantois completely fills the extra-embryonic coelom and becomes attached to the chorion. It is not until this connection has been established and the chorion vascularised that the villi of the chorion commence to develop.

The above account of the early development of the foetus appears to agree in all essential points with that given by Assheton (11) but this author states that attachment commences to take place at the 17th day. It is true that the characteristic changes in the uterine mucosa are initiated at about this time but the interdigitation of the two tissue does not commence until about the 26th day.

II : CHANGES IN THE OVARIES.

When a ewe is served by a fertile ram and conceives, the corpus luteum spurium becomes the corpus luteum verum or corpus luteum of pregnancy. There is evidence to show, however, that the corpus undergoes slight degenerative changes at about the fifteenth day of pregnancy, i.e., at about the time after oestrum when the spurious corpus luteum begins to atrophy in non-pregnant animals. The ewe killed at the Institute 14 days after service, and five ewes from which genitalia were obtained at the abattoir and found to contain foeti 14 to 16 days old, all showed markedly anaemic and slightly atrophic corpora lutea in their ovaries. The ewe killed on the 18th day, and ewes killed at similar or later stages of pregnancy at the abattoir and at the Institute all showed corpora lutea markedly more vascular than corpora lutea spuria. The seemingly atrophic corpora lutea of the ewes killed at the 14th to 16th day have not yet been examined microscopically.

The corpus remains richly vascular and of about the same size as the spurious corpus luteum until about the 18th week of pregnancy. Thereafter it loses its deep red colour, changing to pale yellow, and atrophy sets in. At the time of parturition the corpus is markedly reduced in size

and/

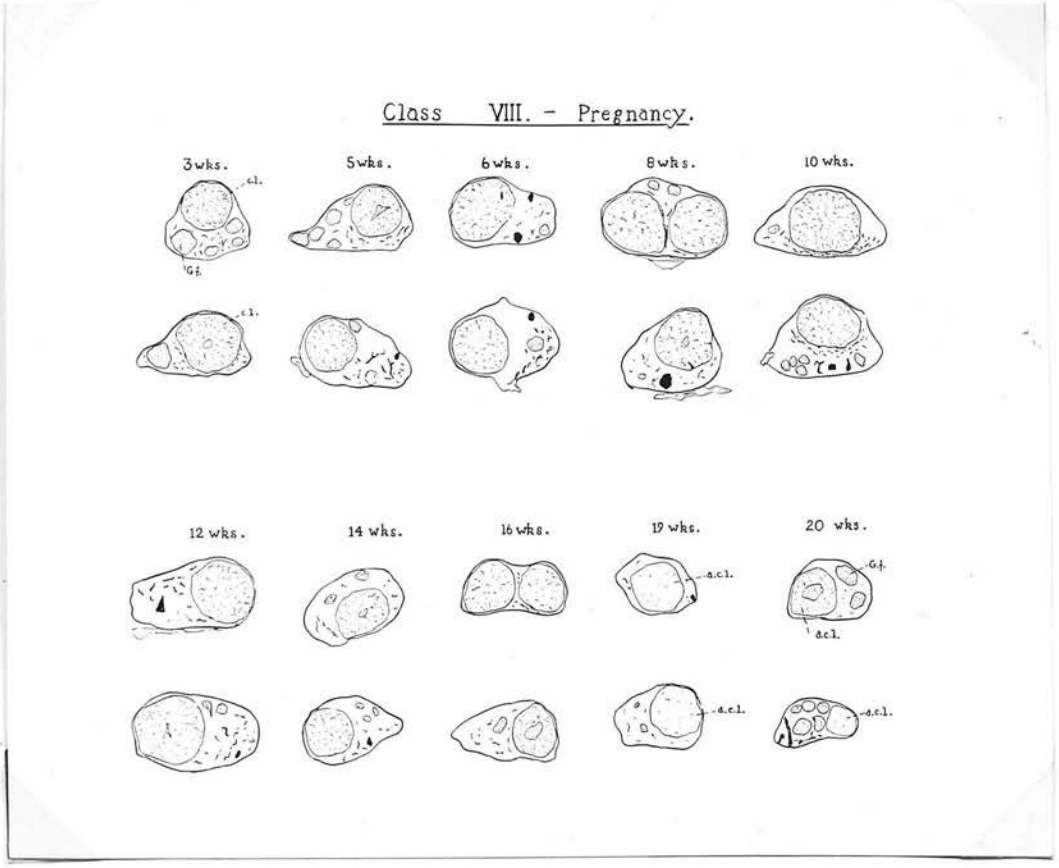


Fig. 51. Sections through ovaries of ewes killed at successive phases of pregnancy. (Diagrammatic)

G.f.	Graafian follicles.
c.l.	corpora lutea.
a.c.l.	atrophic corpora lutea.

and of a deep yellow colour. A series of ovaries removed from Merino ewes during successive stages of pregnancy and lactation have been figured and described by Quinlan and Maré (130).

Apart from the corpora lutea the ovaries undergo gradual atrophy during pregnancy. Until about the 6th week follicles of fair size are found but they rarely exceed about 6 mm. in diameter: ripe follicles or fresh corpora lutea have not been found. After about the sixth week large follicles become increasingly rare and during the fourth month it is usual to find only minute follicles. There appears, also, to be some atrophy of the ovarian stroma for the reduction in size of the ovaries seems to be too great to be accounted for by reduction in the size of the follicles alone.

The atrophy of the corpora lutea is accompanied by an increase in the size of the Graafian follicles.

In the sheep as in other mammals therefore, the corpus luteum seems to inhibit the growth of Graafian follicles. This inhibition seems to be complete in the sheep, for recently formed corpora were never found in pregnant ewes killed at the abattoir. Smith (154) has recorded cases of apparent superfoetation in the ewe but there is some doubt as to the authenticity of these cases.

III : CHANGES IN THE UTERINE MUCOSA.

It has already been remarked that the foetal membranes become attached to the cotyledons only. Normally, nearly all the cotyledons of both horns are utilised whether the pregnancy be multiple or single: only a few of the small cotyledons in the tip of each horn are not involved. Rarely, however, the membranes of a single foetus do not extend to both horns. In the cow adventitious attachements are formed in the intercotyledonary areas late in pregnancy, but these are not found in the ewe.

The attachment of the membranes becomes much closer than in other ruminants. In the cow, for example, the foetal membranes may be separated from the uterus by gentle pulling, even when the placenta is fully developed: this is not possible in the ewe. The form and dimensions of the fully developed cotyledon are shown by Fig. 52, and the structure of the cotyledon is shown in section in Fig. 53.

The placental barrier is of the syndes-:mochorial type, that is, maternal endothelium, maternal connective tissue, foetal epithelium (trophoblast), foetal connective tissue and foetal endothelium intervene between the maternal and foetal blood streams, only the maternal epithelium being lost. The placenta is thus of a relatively

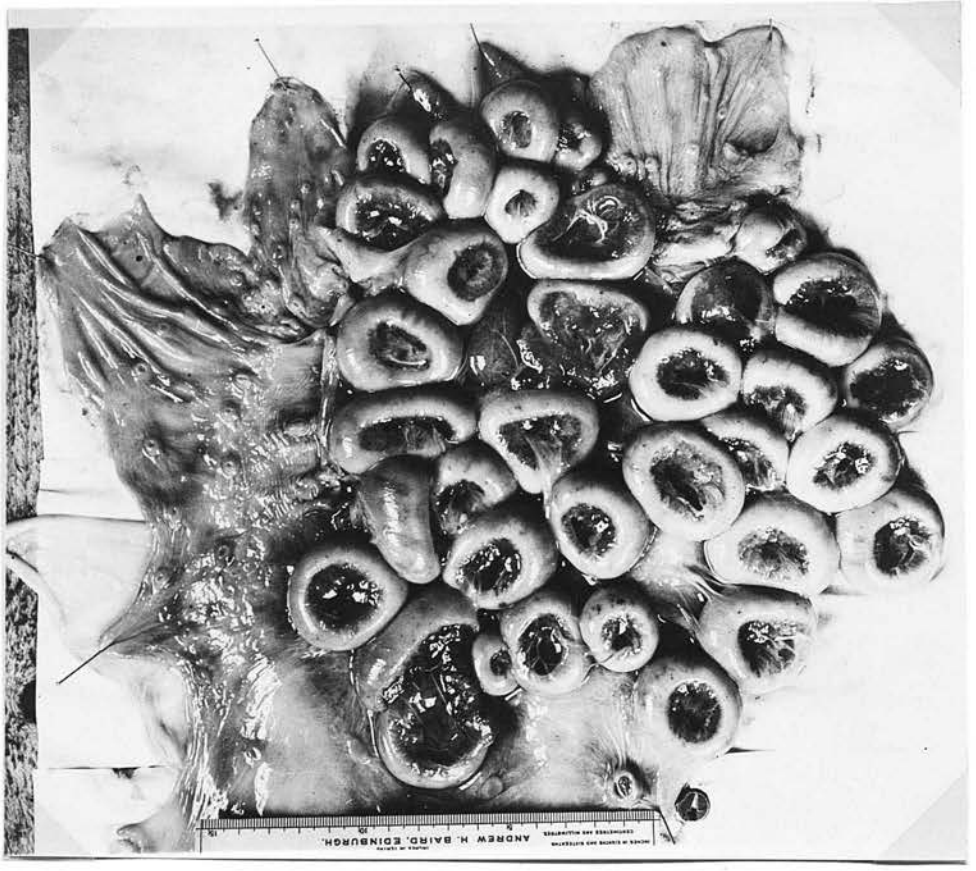


Fig. 52. Placenta of a ewe killed during the fourth month of pregnancy (foetal membranes cut away). In this case the foetal membranes were not attached to the non-pregnant horn and in this the cotyledons are no larger than in a non-pregnant uterus, although they suggest the form of the functional cotyledons.

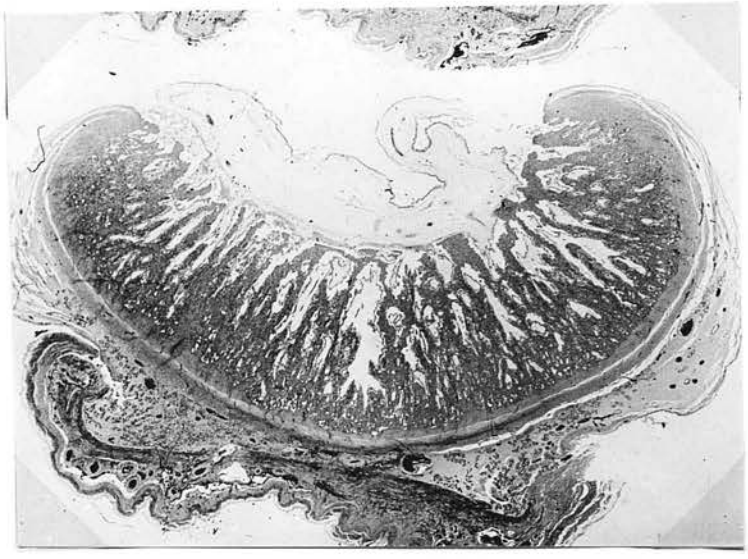


Fig. 53. Section through a fully formed cotyledon from a ewe 3 months pregnant.
x 5.

primative type.

Structural modifications of the uterine mucosa attributable to the presence of the foetus first become evident about thirteen days after insemination. At this time the surface epithelium begins to degenerate in the vicinity of the blasto-:cyst. The uterine glands enter upon a phase of secretory activity more intense than the oestrous phase, their lumina becoming dilated. At about the 15th day the whole mucosa, but that of the cotyledons in particular, becomes greatly congested and the loose stroma becomes oedematous. The slight atrophy of the corpus luteum which takes place at about the 15th day is associated with degeneration of the epithelia of the surface and of the glands. By the 18th or 19th day, when the corpus luteum has re-:covered its normal size and is fully vascularised, the foetal membranes extend throughout both horns of the uterus and the development of the allantois brings the membranes into close contact with the mucosa of the cotyledons. Denudation of the coty-:ledonary epithelium commences about the 18th day and is completed by the 20th day. There can be no doubt that this denudation is brought about by phagocytic or digestive action of the foetal trophoblast.

The intercotyledonary epithelium, after undergoing/

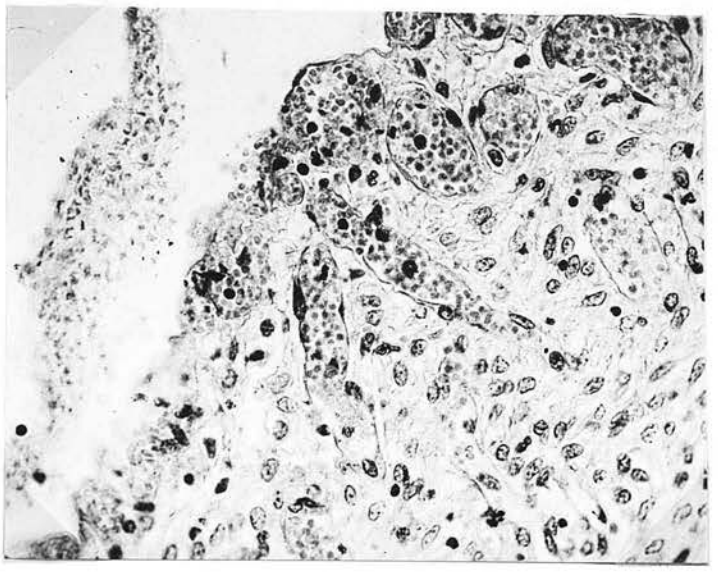


Fig. 54. Sections through surface of a cotyledon of a ewe 21 days pregnant showing denudation of the epithelium, great congestion, and haemorrhage. x 350.

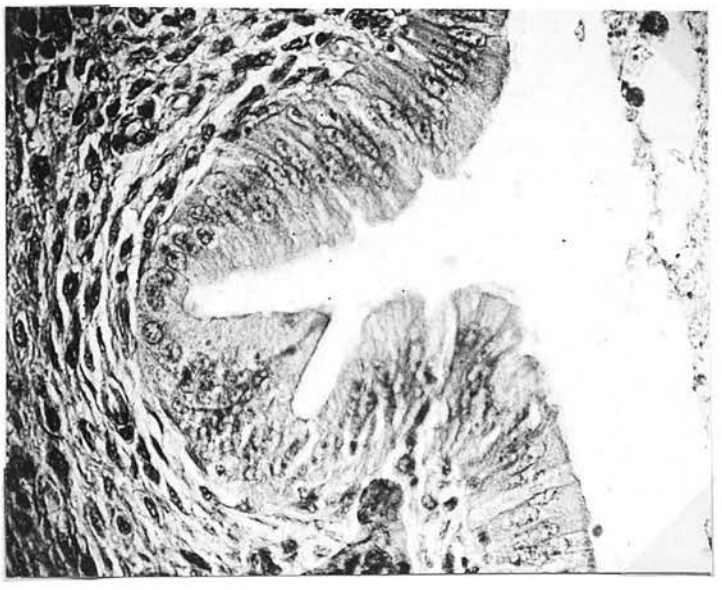


Fig. 55. Epithelium in tip of uterine horn of a ewe 36 days pregnant, showing hypertrophy. Compare with Fig. 18. x 350.

undergoing considerable degeneration at the 15th to 16th day, recovers and beings to hypertrophy. At the end of the first month the intercotyledonary epithelium reaches a remarkable height, especially in the tip of the horn where the uterus is not distended (Fig. 55). At about the 25th day the intercotyledonary epithelium begins to show intense secretory activity: this continues throughout pregnancy. In the later stages of pregnancy the cells are reduced to cubical form, probably mainly as a result of distension of the uterus.

The glandular epithelium also hypertrophies after a degenerative phase at the 15th to 16th day, but this hypertrophy is largely offset by the distension of the glands and the intense secretory activity which sets in at about the 25th day. Dilation and secretory activity continue throughout pregnancy. By the third month the glands are so large that they are readily seen by the naked eye in gross sections through the mucosa and the gland mouths form conspicuous depressions on the surface of the mucosa (Fig. 56).

Secretion from the uterine mucosa and glands takes two forms, as in the dioestrous cycle, i) the discharge of substances elaborated by the cytoplasm of the epithelial cells, and ii) extrusion of cells or cell nuclei from the epithelia. The secretions produced/

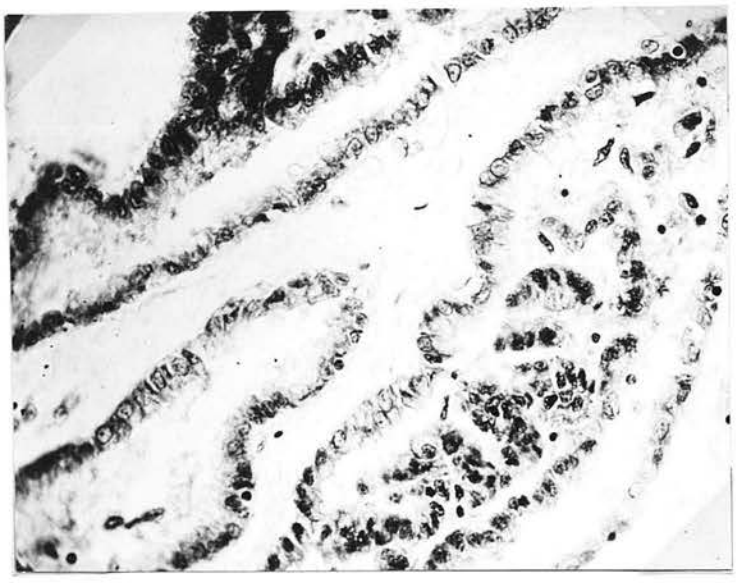


Fig. 56. Uterine glands of a ewe killed in mid-pregnancy, showing dilation, secretory activity and degenerate epithelial cells in gland lumina. Compare with Figs. 22 and 23. x 350.

produced by these epithelia constitute at least the bulk of the "uterine milk", that is, the nutritive fluid found between the foetal membranes and the uterine mucosa, which must be alone responsible for the nutrition of the foetus before attachment and is perhaps the major source of nutriment even when the placenta is fully developed.

Mitotic division of the epithelial cells of the surface and glands continues throughout pregnancy but is never frequent: it never reaches the level that is common during early metoestrum. It is more common in the deep gland tubuli than elsewhere.

The whole uterine mucosa, but that of the cotyledons in particular, becomes greatly congested after about the 15th day. At about the 20th day the capillaries near the surface of the cotyledons usually break down and, since the epithelium is denuded, blood escapes into the space between the uterine mucosa and the foetal trophoblast. The fact that extravasation does not take place in the deep stroma or in the intercotyledonary areas may be taken as an indication that the breakdown of the capillary walls is due to the action of the foetal trophoblast. Congestion subsides somewhat after the foetal mem-:branes have become attached to the cotyledons but the uterus remains greatly congested throughout pregnancy.

There/

There is commonly, but not invariably, a great increase in the number of lymphocytes in the mucosa at the 20th to 30th day. Polymorphonuclear leucocytes are sometimes found in small numbers during pregnancy although they have not been seen in any ewe killed during the dioestrous cycle.

It has been seen that the foetal trophoblast is composed of phagocytic cells which probably also secrete digestive substances and are in all likelihood responsible for the destruction of the uterine epithelium, superficial stroma and capillary walls. The fixation of the foetal membranes is not due entirely to the burrowing action of the trophoblastic villi, however: fixation appears to be a reciprocal process in which both foetal membranes and uterine mucosa take part. The primary stimulus appears to come from the foetus: at about the 24th to 25th day after coitus there are faint indications of the development of the foetal villi, but the maternal stroma is quite unaltered in form. At the 27th day the foetal villi were found to be better developed and in addition the superficial cells of the stroma between the villi were enlarged and stained more intensely, and the intercellular spaces were considerably greater. The foetal villi continue to eat their way in, and by about the 40th day they reach the base of the cotyledon, the stroma cells/

cells having become, by this time, almost completely converted into the large, darkly staining type of cell. The changes in the maternal stroma cells are thus analagous to those which take place in the formation of the decidual cells of the placenta in the rat, etc.; indeed, the modified cells in the sheep are true decidual cells for they are cast off after the foetus has been discharged.

In the rodents the formation of the decid-:ual cells is independent of the presence of the foetus, for it may be induced by stimulation of the uterine mucosa by means of a foreign body and in certain circumstances these cells are formed with-:out stimulation of any kind. In the sheep, on the other hand, decidual cells are not formed unless the uterus is in contact with and distended by, the allanto-chorion. Mere contact with the chorion is not sufficient for when the allantois does not extend into the non-pregnant horn of a unilaterally pregnant ewe the cotyledons remain unchanged, although the chorion is present. Similarly, decidual cells are not formed in the tips of the horns where distension does not take place.

The growth of the maternal part of the placenta is associated with proliferation of the stroma cells. Mitotic figures are found in the stroma throughout pregnancy. But in the primary development/

development of the placenta proliferation does not play a very important part: the growth is effected mainly by the alteration in the type of cell and the development of intercellular spaces.

Throughout pregnancy a foetal epithelium composed of very large, actively phagocytic cells is in close contact with the decidua. Whether or not this membrane is the original foetal trophoblast is uncertain for no other membranes intervene between it and the allantoic blood vessels. The chief products of the maternal tissues phagocytosed by this membrane are fat globules, erythrocytes, decid-:ual cells and the peculiar "stäbchen" already referred to. These last appear to remain in the epithelium since they are present in great numbers in the later stages of pregnancy while the maternal tissues contain very few.

The phagocytic epithelium covering the villi is continuous and identical with that which is in contact with the general uterine surface. In the latter situation it is concerned with the absorption and phagocytosis of the formed elements of the "uterine milk", that is of fat globules, lymphocytes and epithelial cells cast off from the uterine surface and the uterine glands. Bonnet (28) states that leucocytes are one of the more important constituents of the uterine milk but the present writer found polymorphs/

polymorphs in only a few smears from the uterine mucosae of pregnant ewes, and lymphocytes, while they occurred in all, were present in very small numbers only.

Zeitschmann (169) states that the volume of uterine milk in the cow is three-quarters of a litre and in the goat one-quarter of a litre. In the sheep the amount of this fluid is rarely more than sufficient to wet the uterine mucosa thoroughly. Its maximum volume probably does not exceed 10 c.cs.

IV : CHANGES IN THE CERVIX.

The changes taking place in the cervix during pregnancy are simply a gross exaggeration of those occurring during the metoestrous and dioestrous phases of the oestrous cycle, while those occurring at parturition are essentially the same as those which occur in oestrum.

During the whole duration of pregnancy mucus secretion continues to take place in the cervix. The mucus produced is of the stringy, tenacious type and only very little of it escapes into the vagina. Consequently, the cervical canal becomes completely filled and ultimately considerably distended by the mucus in its lumen. This distension takes place against the resistance of the cervical muscles and the effects of this pressure are shown very clearly by the mucosa: many of the lamellae are reduced to vestiges by the stretching of the mucosa, and those which remain become greatly flattened (compare Figs. 57 and 58). The epithelium becomes greatly flattened and disorganised and the nuclei shrunken (Fig. 59). In the last month of pregnancy many of the cells have ceased to secrete mucus.

THE CERVIX IN PARTURITION AND THE PUERPERIUM.

It has been noted that in the last month

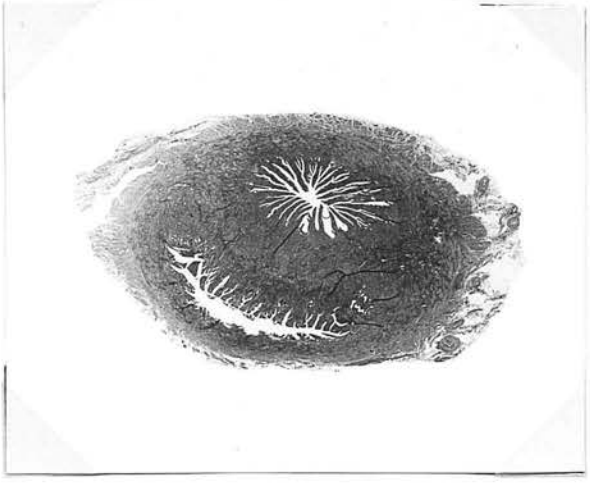


Fig. 57. Section through cervix of a ewe killed in anoestrum. Note absence of mucus from lumen and thickness of laminae. x 6.

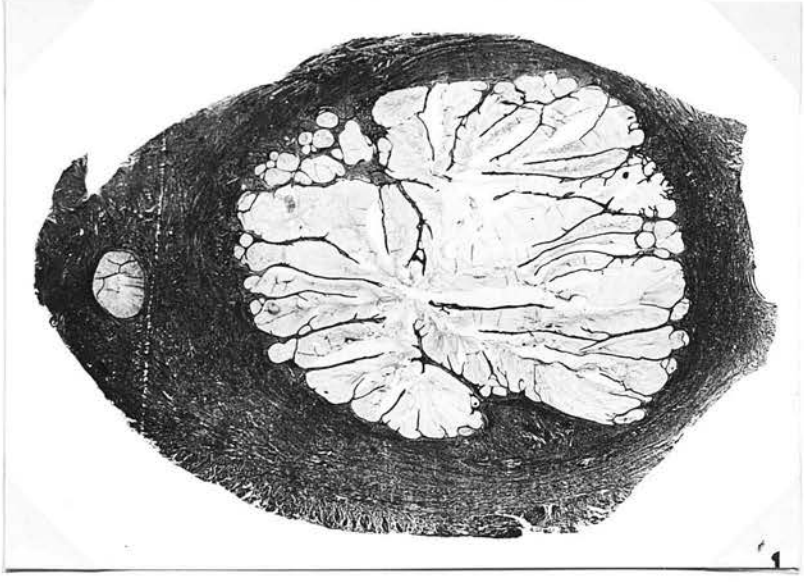


Fig. 58. Section through cervix of ewe killed in last month of pregnancy. Note distension with mucus and flattening of the laminae by the pressure of the mucus. x 6.

Fig. 59. Showing compression and distortion of cervical mucosa by the pressure of the mucus in the cervical lumen in late pregnancy. Stained with Heidenhain's haematoxylin and Mayer's mucicarmine. x 400.

of pregnancy the cervical canal is completely filled and distended by a plug of tenacious mucus. About a week before parturition this mucus begins to liquefy: shortly before labour begins great quantities of mucus flow into the vagina and escape from the vulva. The mucus is thin and watery and similar to the mucus flow of oestrum. The cervix also relaxes greatly.

The passage of the lamb causes considerable destruction of the epithelium and mucosa and haemorrhage usually takes place. The reorganisation of the epithelium during the puerperium is quickly effected and three weeks after parturition it is fully restored. The lamellae do not develop to their full size until some months after parturition. Small quantities of mucus sometimes appear in the reorganising epithelium but not more than is commonly found in anoestrum. Conditions are presumably different in those breeds of sheep in which oestrum recurs during lactation but such have not been studied.

The sub-epithelial accumulations of blood resulting from the parturition haemorrhages grad-:ually become converted into masses of amorphous brown pigment, the nature of which has not been determined.

V : CHANGES IN THE VAGINA.

Pregnancy, in the ewe, is marked by characteristic modifications of the vaginal mucosa and secretions.

1. THE VAGINAL SECRETIONS.

When a ewe becomes pregnant the cyclical alterations in the character of the vaginal smear cease to occur, and after the initial metoestrous desquamation following oestrum the smear assumes a monotonous character, similar to that found during anoestrum. In late pregnancy the mucus content rises considerably and at parturition great quantities of mucus are poured into the vagina as a result of the liquefaction of the cervical plug. The total cellular content of the fluid is very small, about equal to that which is found during the true an-:oestrum. The cell types present are healthy vaginal epithelial cells, polymorphonuclear neutrophilic leucocytes and a few lymphocytes. To-:wards the end of pregnancy eosinophilic granular leucocytes may appear. Leucocytes formed a constant constituent of the smears taken from experimental ewes through pregnancy but they were found in only 13% of the smears taken from pregnant ewe genitalia obtained at the abattoir. The mean percentage concentration/

concentration by number of leucocytes in slaughter-house smears from pregnant ewes was only 3% - the same figure as that obtained for ewes killed in anoestrum. Comparative figures for leucocyte concentration in the various reproductive states have already been given (Table IX on page 247).

Semi-cornified vaginal epithelial cells were found in some smears from ewes killed in an-:oestrum and in the several stages of the dioestrous cycle but they were never found in smears from pregnant ewes after the first month.

While there are thus notable differences between the vaginal smears from anoestrous and preg-:nant animals, these differences appear only when groups of ewes are compared. They cannot therefore, be used as a diagnostic measure for the detection of pregnancy in any one individual.

2. THE VAGINAL MUCOSA.

During the last month to six weeks of pregnancy the vagina becomes greatly congested, this congestion becoming increasingly obvious as parturi-:tion is approached. There is rarely any haemorrhage or extravasation of blood except at parturition, however. Structural modifications of the sub-epithelial mucosa are very slight. Oedema occurs during/

during late pregnancy, probably as a result of congestion. The number of lymphocytes does not change appreciably.

It has already been noted that the cells of the superficial layer of the vaginal mucosa may become converted into tall columnar "secretory" cells during pregnancy. Whether these cells have actually any secretory function is doubtful. This modification is most commonly found near the os externum of the cervix: the vestibule rarely shows these cells. While the columnar type does not always occur the whole epithelium is always covered by a much larger, more cubical type of cell than is usually found during anoestrum or the dioestrous cycle and, as already mentioned, the extremely flattened, semi-cornified type of cell is never found.

V : D I S C U S S I O N.

It has been found desirable to include much that might have been placed under the present heading in the body of this Thesis. The significance of the breeding season and the factors which control it have, in particular, been discussed at some length. Certain more general aspects of the study relating to the phenomena of the dioestrous cycle remain to be discussed, however.

The Cause of Heat.

In almost all mammals, under normal conditions, exhibition of the mating instinct is associated closely with the presence in the ovaries of mature Graafian follicles, and the cessation of heat with the disappearance of these follicles, either by rupture or by atresia. In the ferret, in which animal rupture of the follicle depends upon the stimulus of coitus, the oestrous state persists for several months in the unmated animal and ceases only when the follicles in the ovary begin to undergo atresia (Hammond and Marshall (67)). A similar condition exists in the rabbit. The phenomenon of nymphomania in the cow, mare, etc., is usually associated with the presence in the ovaries of un-:ruptured follicles which have become cystic.

The closeness of the relationship existing between heat and large follicles led to the view that heat/

heat was actually due to a stimulus emanating from the ripe follicle. Pflüger, Strassmann and others (cit. Marshall (106)) thought that menstruation in the human and heat in animals were due to nervous reflexes originating from the pressure of the ripening follicle upon nerve endings in the ovary: this view has been discredited by experiments in which the spinal cord was transected without influenc-:ing the occurrence of heat. Moreover, it has been shown that ovaries transplanted to abnormal positions are adequate to maintain oestrus and also that heat can be induced by the injection of extracts of ovarian substance, particularly of the liquor folliculi, into spayed animals.

It has now been established beyond reason-:able doubt, therefore, that one link in the chain of factors causing oestrous is a chemical substance elaborated or concentrated by the ovary. This "oestrogenic" substance, usually called "oestrin" in this country, has now been isolated in crystaline form and much is known of its chemical constitution; but the precise site of production of this substance and the nature of the stimuli which induce its formation are still problematical. Since heat is normally associated with the presence of large follicles and the concentration of "oestrogenic" hormone is far greater in the liquor of the ripe follicle/

follicle than in other parts of the ovary, the most obvious site of origin would appear to be the granulosa or thecal cells of the ripening follicle: there is, indeed, good reason to suppose that in the majority of mammals under normal conditions the ripe follicle is at least the chief source of the hormone. But there are many facts which show that the presence of ripe follicles is not entirely necessary for the elicitation of heat and therefore, presumably, that the ripe follicle is not the only source of "oestrin". Thus it has been shown that in many bats mating takes place in the autumn, while the maturation and rupture of Graafian follicles does not ensue until the fol-:lowing spring. In the human female, and probably in some other Primates, the mating desire is constantly present although large Graafian follicles are present only during a small part of the menstrual cycle. Many authorities hold that the peak of sexual desire in women occurs during the pre-:menstrual period (Davis (49)) while the development of the follicle occurs near the mid-interval. Various methods of experimental interference also, have shown that oestrum is not necessarily dependant upon the presence of ripe follicles. Parkes et al. (126, 124, 125, 35, 36)have shown that in the mouse the germinal tissue may be destroyed by means of X-rays and the development of Graafian follicles thus be/

be prevented without influencing the sex cycle appreciably. It has been shown also that oestrus may occur 36 to 48 hours after ovariectomy so that the oestrous stimulus appears to be active two days before heat occurs, i.e., before follicular maturation commences (33).

In the guinea-pig Genther (62) found that oestrus occurred after X-ray treatment only when large follicles were present in the ovaries, i.e., after only partial destruction of the follicular tissue. The ferret seems to be intermediate between mouse and guinea-pig in this respect for in this animal (128) complete X-ray sterilisation abolishes the mating instinct and the vulval changes without greatly affecting the uterine changes which take place at oestrum.

The other ovarian tissue which it is thought may be involved in the production of the oestrous hormone is the so-called "interstitial" tissue. The amount of this tissue which is present in the ovary varies enormously from one species to another and it may be that this is the reason why different species react so differently to follicular destruction.

It is probable that the membrana granulosa of the follicle does take part in the production of the hormone in all species but it is possible that the/

the high concentration in the liquor folliculi is due simply to passive concentration in a non-circulating fluid of a substance elaborated in another part of the ovary.

It has been shown that in the sheep normal maturation and rupture of Graafian follicles occur regularly prior to the commencement of the normal breeding season, and rarely at other times, without the exhibition of the mating instinct. So far as the writer is aware this phenomenon has not been observed in other animals. On the other hand, heat is well known to occur in many mammals without ovulation, the mature follicles going into atresia. That some oestrous hormone is produced at these spurious cycles in the sheep is indicated by the occurrence of the secondary oestrous phenomena in the vagina and uterus. It is reasonable to conclude, in the absence of experimental data, that the ovary does not produce a sufficient quantity of oestrin during the spurious cycles to raise the concentration in the blood above the "heat" threshold. Another possible explanation is that the receptor and not the effector system is at fault; that oestrin is produced in adequate amounts but that the psychological phenomenon is not elicited. Adequate methods are now available for the detection and estimation of oestrin and the question may be tested experimentally. There is/

is no apparent lack of the substances necessary for follicular maturation (anterior pituitary "gonadotropic" hormone or Heape's "generative ferment") during the spurious cycles for multiple ovulations are just as common during those cycles as when heat is expressed. This also, however, is a matter which may readily be checked experimentally.

While the causation of heat thus rests ultimately upon the secretion of an oestrogenic substance by the ovaries, nothing whatever is known of the manner in which this substance evokes the psychological phenomenon of mating desire. The action may be a direct one upon a brain centre or it may involve a chain system acting through a nerve path not yet discovered. Evidence is accumulating to suggest that at least one more hormonic link is involved, that is, that oestrin does not stimulate the nervous system directly but induces the formation by another endocrine gland of a substance which is more directly responsible for the neural stimulus. Experiments conducted by Wiesner (167) indicate that in the male rat mating desire is induced by the testicular hormone through a hormonal stimulus to the anterior pituitary gland. The assumption that a second hormonic link is involved in the female simplifies the interpretation of the peculiar "delayed heat" encountered during the present investigation in ewe/

ewe 816. It will be recollected that in this ewe the vaginal changes at the sixth cycle recurred with the normal periodicity, i.e., they occurred 16 days after the previous heat and 16 days before the following heat. The mating phase at the sixth cycle was delayed for fully four days, however, i.e., the intervals between heat periods were 20 and 12 days instead of 16 and 16. The occurrence of the vaginal phenomena may be taken to indicate that follicular maturation and the secretion of oestrin occurred at the normal time and it is difficult to understand how the lag could have occurred if oestrin stimulates the nervous system directly. If another endocrine gland is concerned the delay is more readily under-:standable.

It is possible, also, that failure of this hypothetical second hormonic link may be responsible for the non-exhibition of the mating desire at the early periods of follicular maturation and rupture.

In the human being the mating instincts of the female have been largely subjugated by the brain, but periodicity of sex desire is well known to exist and is presumably due to hormonal factors. It is well known that not only the sex instinct but also the menstrual cycle in women may be affected through the nervous system. Cessation of menstruation can be brought about by hypnotism and by other peculiar mental/

mental states. Distinctive instances of similar phenomena in the lower mammals do not appear to be known but the practice of throwing cold water over a cow in order to cut short the heat period presumably depends upon a nervous stimulus. In birds, the taking away of the eggs may cause the laying of several clutches when only one is ordinarily laid: this change also is presumably effected through the nervous system.

The Cause of Ovulation.

In a great majority of mammals, including the sheep, the ripe Graafian follicle does not per-:sist in the ovary of the unmated animal for any great length of time but either continues to grow and ruptures, or, under unfavourable nutritive or environmental conditions, undergoes atresia. In the unmated rabbit, ferret and cat, on the other hand, the Graafian follicle grows only up to a point, at which it persists for some time and then undergoes atresia: further growth and rupture of the follicle do not take place (occasionally in the ferret) with-:out an additional stimulus. Under normal conditions this additional stimulus is provided by coitus, but it may be replaced experimentally by intravenous injection of anterior pituitary lobe, or human pregnancy urine extracts (Bellerby (15), Friedman (59, 60), Snyder and Wislocki (156), and others).

The/

The path of action of the coital stimulus probably lies through the anterior pituitary gland since removal of the pituitary shortly after coitus prevents the ovulation which would normally follow after about 12 hours, but if the pituitary is not removed until an hour after coitus ovulation is not prevented (Fee and Parkes (54)).

But as to the manner in which the activity of the pituitary is affected very little is known. Fee and Parkes assume that the stimulus is carried through the nervous system since Hammond (65) showed that ovulation could not be produced by artificial insemination, but the same authors showed in a later paper (Fee and Parkes (55)) that vaginal anaesthesia does not prevent ovulation after coitus. The experi-:ment was not conclusive, however, since the copula-:tory reflex was not suppressed: anaesthetisation of the vagina was complete, so that if the stimulus to the pituitary is nervous in origin it probably operates through the general emotional state of the animal and not through a direct nerve path. The fact that actual penetration of the vulva by the male is not necessary to induce ovulation (Hammond (65)) is a further indication that the stimulus is emotional in origin.

That the stimulus of coitus is a nervous one acting directly upon the ovary or eliciting a hormonal stimulation/

stimulation of the pituitary by the ovary is improbable since ovulation following coitus takes place in the transplanted ovary, the nervous connections of which have been severed (Asdell (9), Friedman (59)).

This last fact indicates also that the action of the pituitary upon the ovary is not effected through the nervous system but is of an endocrine nature. There is nothing to indicate that there is a specific ovulation-producing principle of the pituitary: the ovulation-producing extracts of anterior lobe and of pregnancy urine that have been used do not differ from those which produce maturity in immature animals. The experiments of Brambell and Parkes (34) suggest that not only concentration of pituitary hormone but also the actual amount available is a limiting factor in ovulation since, when the concentration of circulating hormone in the body after coitus is considerably reduced (by replacement of some of the blood) and ovulation thereby prevented, if some of the large follicles in the ovary are destroyed the remainder proceed to grow and rupture.

It thus appears that, while in the majority of mammals the activity of the anterior pituitary which occurs at the time of oestrus is sufficient to cause ovulation, in the rabbit and similar animals this threshold is not reached unless copulation takes place./

place. It is very improbable that there is any essential difference in the actual mechanism of ovulation involved.

While it is thus reasonably certain that ovulation is dependant upon the gonad-stimulating hormone of the anterior pituitary lobe and represents the culmination of the action of this substance, it is by no means clear how these effects are brought about. The actual rupture of the Graafian follicle is almost certainly a purely mechanical phenomenon due to the accumulation of fluid within its cavity. Robinson (134) found that there was a rapid swelling of the follicle after coitus and just before ovulation in the ferret. A similar pre-ovulation swelling was found by Hammond (66) in the cow and by Long and Evans (95) in the rat. In the sheep, as has been shown by the author's material, there is a rapid and very considerable swelling of the follicle, immediately prior to ovulation (see page 150). The view expressed by Schochet (145) that ovulation is due, not to mechanical pressure but to the action of digestive substances appearing in the liquor folliculi is now little credited. Investigation has failed to detect digestive ferments in the follicular fluid and histological examination of the turgid follicle gives no evidence of disintegration of the follicular cells.

The/

The thecal layers of the mature follicle are richly vascular and congested prior to ovulation. In the rabbit, in which this increased vascularity of the theca is particularly marked, haemorrhage from the thecal capillaries, especially those at the point of rupture of the follicle, usually takes place prior to actual dehiscence of the follicle. The same phenomenon is frequently seen in the cow (66). That this haemorrhage is the actual cause of rupture is rendered improbable by the fact that it does not take place in the majority of mammals. It never occurs in the ewe. Also, in unmated rabbits, follicular atresia is usually accompanied by haemorrhage into the follicular cavity.

The liquor folliculi is now regarded as mainly a plasma transudate, the cells of the granulo-:sa and theca interna contributing little to its bulk. (Lyons (97)). Whether this is the case or not, the increased vascularity of the follicle prior to rupture clearly favours the accumulation of fluid within the follicle. The immediate effect of coitus upon the vascularity of the follicular theca in the rabbit and ferret does not appear to have been studied.

That the growth in size of the follicle does not in itself bring about rupture of the thecal membrane is indicated by the condition of nymphomania, common/

common in the cow and mare, and due to the persis-:tence of very large follicles in the ovaries.

In the opinion of the writer rupture of the Graafian follicle is probably due to the rapid accumulation of fluid in the follicle as a result of congestion of its theca, the expansion of the follicle being thus too rapid to allow the granulosa and theca interna to compensate for the increase in the follicular contents. If the accumulation of fluid in the follicle is slow, either atresia sets in or the theca is able to compensate for the expansion of the follicle and a cyst is formed.

The Factors Regulating Oestrous Periodicity.

It has been shown that the causation of heat is probably referable ultimately to the ovarian follicular hormone. The mechanism regulating ovarian periodicity in mammals is still highly problematical however. It has been shown conclusive-:ly that the secretion of the corpus luteum inhibits follicular maturation in the rat and mouse, and the fact that follicular maturation is in abeyance throughout pregnancy and during interoestrum in the cow and ewe indicates that in these mammals also the corpus luteum causes follicular inhibition. Hammond (66) has shown that in the cow, removal of the corpus luteum during interoestrum hastens the onset of the following heat period. A few experi-:ments conducted on the ewe suggest that in this animal/

animal also removal of the corpus luteum is followed quickly by the onset of heat. It has been shown, also, that the atrophy of the corpus luteum which commences on about the fifteenth day of the dioestrous cycle is soon followed by the rapid development of Graafian follicles and the onset of heat. It may be stated with reasonable assurance therefore, that the corpus luteum of the ewe, by the inhibitory action that it exerts upon follicular maturation, is immediately responsible for the maintenance of ovarian, and consequently of oestrous, rhythm. The factors which restrict the life of the spurious corpus luteum within very narrowly defined limits are, however, still a matter for conjecture. The atrophy of the corpus on the fifteenth day of the cycle might be due a) to an inherent inability to persist beyond this age, b) to the appearance in the blood stream of a substance responsible for the death of the luteal cells, or c) to the removal of some hormonic substance necessary for the life of the luteal cells. The first of these appears to be eliminated by the fact that the life of the corpus is prolonged for four months if the animal becomes pregnant: also, many workers claim to have prolong-:ed the life of the corpus luteum in non-pregnant rodents by hypophysectomy and also by the continued administration of anterior pituitary lobe substances. Thus/

Thus the life of the corpus luteum spurium may be prolonged under favourable circumstances and it seems improbable that "senility" of the luteal cells is alone responsible for their death. It has been suggested that, in rats, the corpus luteum of pseudo-:pregnancy becomes senile and slight degeneration commences to take place; that this removes the inhibition of the hormonic mechanism controlling follicular maturation and allows the development of follicles and that the secretion of these, in turn, precipitates the degeneration of the corpus luteum. This mechanism cannot apply to the ewe for follicular development does not take place until the corpus luteum has commenced to regress. Also, pregnancy in the ewe is followed by anoestrum and not by a phase of follicular maturation as it is in the rat.

The suggestion that atrophy is due to the production of an inhibiting substance appears to be an improbable solution for it has been shown that in the ewe, atrophy of the corpus luteum sets in on the 15th day of pregnancy but is soon arrested and the corpus recovers: if an inhibitory substance were responsible for this atrophy it would not be expected that the corpus would recover.

The third suggestion, that atrophy of the corpus luteum takes place as a result of a failure in the supply of some hormonic substance necessary for/

for the life of the corpus luteum has least evidence against it and appears to the present writer to be the most probable solution. Even if this relation were definitely proven and the death of the corpus luteum spurium were shown to be due to the disappear-:ance of some substance, for example, a pituitary hormone, from the blood stream and the revival of the corpus in pregnant animals to be due to the reproduc-:tion of this substance, this conclusion would not help materially in elucidating the mechanism control-:ling oestrous periodicity for it would still remain to be shown whether the pituitary or other organ responsible for the production of the substance necessary for the continued life of the corpus luteum possessed its own autonomous rhythm or whether this rhythm was, in turn, imposed upon it by some other hormonic link.

Thus, while the immediate factor control-:ling oestrous rhythm in such animals as the cow and ewe at least is almost certainly the corpus luteum, the more remote links in the chain of factors regulating oestrous periodicity remain obscure and will probably prove excessively difficult to elucidate.

Quinlan and Maré (130) have suggested that prolonged heat periods in the ewe are caused by delayed rupture of the Graafian follicles. While the writer does not subscribe to this view, there is no/

no proof one way or the other. Great interest may be attached to the verification or refutation of the hypothesis, however, for it has been shown that the duration of heat is not correlated with the duration of the dioestrous cycle. Thus, if prolong-:ed heat is associated with delayed ovulation, the life of the corpora lutea formed is curtailed, since heat does not commence until the corpus of the prev-:ious cycle has begun to atrophy. If this relation holds therefore, "senility" of the luteal cells cannot be responsible for their death at the 15th day and the periodicity of the oestrous cycle must be determined by some organ other than the ovaries and probably depends upon the rhythmic secretion of a substance which inhibits the life of the luteal cells, or inhibits the production of a substance necessary for their life.

The Changes in the Accessory Reproductive Organs.

The modifications of the mucosae of the Fallopian tubes, uterus, cervix and vagina which accompany the dioestrous cycle are open to three possible functional interpretations: a) they might assist coitus and protect the female organs from injury or infection, b) they might be directed towards providing a favourable environment for the gametes, or c) they might represent the commencement of changes directed towards the provision of a favourable environment/

environment for the foetus, these changes serving a useful end only if the ovum liberated in oestrum is fertilised. In the ewe, the vaginal and cervical changes may be functional in coitus but the changes in the uterus and Fallopian tubes appear to play no part in this act for uterine secretions do not escape into the vagina and there are apparently no co-ordinated orgasmic contractions of the uterus serving to suck up the sperm, such as have been found to exist in the rat (69).

It has come to be recognised generally that the hypertrophic changes which occur in the uteri of the non-pregnant rabbit, bitch and human being for example, during the period when active corpora lutea are present in the ovaries, are caused by a secretion of the luteal cells and may be regarded as identical, except in intensity, with those which occur during pregnancy. Since corpora lutea apparently almost identical with those found in pregnancy, are found in the ovaries of the ewe during the greater part of the interoestrous period, it might be anticipated that the changes in the uterus during interoestrum would be homologous with those which occur during pregnancy. It will have been seen from the descriptions that have been given of these changes, that there are definite homologies between the changes during the luteal phase of the oestrous cycle and/

and those which occur in pregnancy, but that the similarity is not striking. During interoestrum the epithelia of the uterus and uterine glands hypertrophy. The hypertrophy continues in the epithelium of the surface where this is not in con-:tact with the foetal membranes and in some of the glands during the first two months of pregnancy. But these epithelia display only slight secretory activity during interoestrum whereas they show intense secretory activity throughout pregnancy. The stimulus to continuous secretory activity seems to originate from the foetus but it is doubtful whether the foetus actually initiates these changes, for it has been found that secretory activity (other than in the immediate neighbourhood of the foetus) does not commence until about the 15th day after service, i.e., about the time when secretory and degenerative changes commence in a non-pregnant animal. In a pregnant ewe, as in a non-pregnant animal, the commencement of secretory changes is associated with commencing atrophy of the corpus luteum. When it is remembered that the development of the foetus is very slow until about the fifteenth day and that thereafter growth proceeds with remark-:able rapidity, it appears probable that the presence of the foetus does not affect the normal dioestrous cycle until the corpus luteum begins to atrophy. When/

When this occurs the secretion and degeneration of the epithelium provide the foetus with the materials for rapid growth: this growth, probably by stimulating the uterine mucosa, arrests the atrophy of the corpus luteum, but the high, non-secretory uterine epithelium is not restored where the foetus is in contact with the mucosa, although it takes place and is followed by further hypertrophy in the tips of the horns where the foetal membranes are not present.

This view is stated with reserve, however, for it is not yet certain that degenerative changes do actually commence in the corpus luteum at the 15th day of pregnancy and it may be simply that the natural tempo of growth of the foetus is slow until about the 15th day and that the secretory and degenerative changes are initiated by the commencement of rapid growth at this time. Nevertheless, continu-:ed secretory activity in the surface epithelium during pregnancy must be determined by contact with the foetal membranes for otherwise there is no explanation of the continued hypertrophy without much secretion in parts of the epithelium not in contact with the membranes.

While the changes in the epithelia of the uterus during interoestrum may thus be regarded as equivalent to the changes which occur in pregnancy and/

and are therefore analogous to the "progestational" or "pseudopregnant" changes in the rabbit and bitch, it is not proposed to apply the term "pseudopregnancy" to these changes, for, while the author is unaware of any records of such phenomena in the sheep, in the cow true pseudopregnancies marked by long cessation of heat, mammary enlargement, etc., do sometimes occur, and it seems advisable to restrict the term to this type of change. If the alternative view be taken, however, and the dioestrous cycles of the ewe and cow are regarded as pseudopregnant cycles, then an ordinary dioestrous cycle such as that which occurs in the rat and mouse in the absence of mating does not occur in these species and probably not in the other Ungulates.

Although the changes in the uterine mucosae of ruminants during interoestrum are analogous to the "pseudopregnancy" changes in rodents there is very little homology between the phenomena in the two groups; nor is there any reason to expect such homology for the changes during pregnancy are markedly different. There can be little doubt that, from the evolutionary standpoint, the primitive uterus was a slightly modified oviduct which probably first harboured, and later produced nutritive substances for the nourishment of, the growing embryo. The further development of the secretory function would lead/

lead to the increasing of the productive surface by folding or the development of crypts. In Ungulates there has been, comparatively, very little departure from this primitive type, for the foetus is very largely dependant throughout intra-uterine life upon the nutritive substances actively secreted by the uterine mucosa. In the sheep, the foetus is entire-:ly dependant upon these secretions for a fifth of the total duration of gestation, and, even when the placenta is developed, the function of this organ appears to be mainly that of absorbing secretions produced by the maternal tissues: the wide separa-:tion of maternal and foetal blood streams renders it probable that only the more readily diffusable substances are transferred directly from maternal to foetal blood streams, and perhaps the only important exchange effected in this manner is in blood gases. Unfortunately, practically nothing is known of the properties of the "placental barrier" in ruminants. The enormous development of the trophoblast and later of the allantois, and the early age at which these structures develop, point to the great importance of the absorptive functions of these organs and to their great phylogenetic age. The late development of the foetal attachments would appear to indicate that these are a secondary concern not developed phylogenetically until late in the evolution of the animal. It must be admitted, however, that it is difficult/

difficult to account, on this basis, for the early ontogenetic development of the specialised regions of the uterine mucosa which serve for foetal attach-:ment. The cotyledons are developed, in form if not in structure, during foetal life.

In such forms as the rat, on the other hand, the secretory properties of the uterine mucosa are probably of little importance to the foetus. There is no primary expansion of the blastocyst such as that which occurs in Ruminants (and other Ungulates) and the embryo becomes entirely embedded in the mucosa, no part of the foetus or of its membranes remaining in the uterine lumen. The formation of the placenta involves enormous development of the uterine stroma and in the fully developed placenta the foetal villi are bathed in the maternal blood. The secretions of the uterine epithelia are thus of little importance and the foetus is mainly dependant upon the absorption of substances directly from the maternal blood stream.

The formation of the placenta and the relative importance of secretory activity vary enormously in different groups of mammals and also within the several orders but it is doubtful whether in any of the higher groups the secretory activity of the general mucosa assumes the degree of import-:ance that it probably possesses in the Ungulates. Certainly, the methods of providing for the foetus exhibited/

exhibited by the Ungulates on the one hand and by the Rodents on the other are widely divergent and it is no more possible to compare in detail the uterine phenomena of the two groups than it would be possible to submit the digestive processes of the two groups to minute comparison.

In comparison with the other Ungulates, the ruminants form a rather highly specialised group so far as provision for the foetus is concerned: the cotyledonary placenta may almost certainly be regarded as a specialised modification of the diffuse placenta found in the mare and sow. In the latter animals, particularly the sow, the secretory activity of the uterine mucosa is perhaps even more important than in the sheep. The only other Ruminant the reproductive phenomena of which have been subjected to detailed examination is the cow (Hammond (66) and subsequent investigators) and in this animal the structural modifications of the uterine mucosa appear to be almost identical with those in the ewe.

Knowledge of the nature and mechanism of formation of the "uterine milk" of Ruminants has advanced little since the investigations of Bonnet (28, 30, 31) and Kolster (83, 84) were carried out. Camgee (61) found that the uterine milk of the ewe contained 9.9% protein and 1.2% fatty matter. He obtained/

obtained the fluid for analysis from the cotyledons however, and there is no reason to believe that this fluid has the same composition as the true uterine milk, which is produced by the intercotyledonary areas. The main constituent of the uterine milk is probably the secretion produced by the epithelia of the uterine surface and glands. The fluid part may be largely a lymph or serum transudate however. Epithelial cells and products of their liquefaction form an important part of the secretion.

There is no justification for including the fluid which accumulates between the foetal villi and the decidual cells of the cotyledon under the term "uterine milk" for this fluid derives nothing from secretory epithelia. It is probably simply a plasma and lymph exhudate containing disintegrated decidual cells, erythrocytes, etc. Its function is no doubt the same as that of the uterine milk however.

While most of the changes in the uterine mucosa during interoestrum may reasonably be ascribed to the presence in the ovaries of corpora lutea, the interpretation of the oestrous changes is open to more speculation. In the mammalian species generally three types of uterine degeneration are recognised,
i) pro-oestrous degeneration - as in the bitch,
ii) pseudopregnant degeneration - as in the rabbit, ferret, bitch, etc., after pseudopregnant hypertrophy and/

and iii) menstruation in Primates, which many authorities believe to be identical with (ii) but about which there is still much doubt: Hartman, Firor and Geiling (70) ascribe the causation of menstruation to a hormone of the anterior pituitary gland. However, there can be no doubt that pro-oestrous degeneration is distinct from both pseudo-pregnant and menstrual degeneration: Heape's original theory, that menstruation and pro-oestrum are homologous, no longer receives credance. In many Primates both degenerative phases are found, and traces of a pro-oestrous degenerative secretion are sometimes found in women who experience the inter-:menstrual pain thought to be associated with the ripening of the Graafian follicle.

It is difficult to conclude as to whether the oestrous degeneration occurring in the ewe is actually a pro-oestrous degeneration or a pseudo-:pregnant degeneration following upon degeneration of the corpus luteum of the previous cycle. It was hoped to decide this by examining the uteri of ewes killed at the first oestrous period of the breeding season, but the slight nature of the oestrous degeneration and the great individual and regional variation in its exhibition made it impossible to come to any definite conclusion. Many authorities (e.g., Allen (3)), proceeding on the basis of the fact/

fact that continued injection of oestrin causes hypertrophy of the mucosa in rats and monkeys and that cessation of injection is followed by degenera-:tion, believe that the oestrous or post-oestrous degeneration in the uterine epithelium of the rat, mouse, etc., is due to the removal of oestrin follow-:ing bursting of the Graafian follicle. This cannot apply to the sheep, in which the bursting of the follicle does not take place until late oestrum and is immediately followed by restorative changes in the mucosa, nor can it apply to the bitch in which degeneration is a definitely pro-oestrous phenomenon.

In the bitch, there is a very definite pro-oestrous hypertrophy preceding degeneration. This hypertrophy is thought to be due to the secretion of the ripening Graafian follicle. In the sheep no pro-oestrous growth could be detected in individual cycles but there is quite definite hypertrophy during the three weeks or so preceding the first ovulation, and it may be concluded therefore, that the sheep's uterus hypertrophies under the influence of the ovarian follicular hormone.

Hammond (66) concluded that the oestrous or post-oestrous degeneration of the uterus in poly-:oestrous animals represents both the pro-oestrous and the pseudopregnant degenerative phases of monoestrous mammals, such as the bitch, the two being telescoped in/

in polyoestrous animals by the rapid maturation of new Graafian follicles following degeneration of the corpus luteum. The present writer can find no reason to disagree with this view. Careful study of the phenomena associated with the degeneration of the last crop of corpora formed during the breeding season might provide a solution to this problem but the slight nature of the degenerative changes in the ewe would necessitate the examination of very extensive material and cows of a breed which show seasonal oestrus might be more satisfactory for the purpose.

It has been noted that the oestrous degeneration in the sheep is preceded by secretory changes in the uterine epithelium and glands and that these changes are similar to those which commence to occur at about the 15th day of pregnancy, and continue throughout gestation. It is not suggested, however, that the pro-oestrous secretory phase is analogous to that of pregnancy: it is more probable that the secretory phase of pro-oestrum is really the commencement of cytoplasmic degeneration and is not identical with that which occurs during the height of pregnancy, although the commencement of secretory activity during pregnancy may also be due to commencing degeneration for there seems to be slight atrophy of the corpus luteum vera at the 15th to 16th day.

The/

The relation of the wave of mitotic activity which passes over the uterine epithelia to ovarian changes is uncertain. It might readily be concluded, on the basis of conditions in the rodents, that mitosis is due to the secretion of the corpus luteum but the fact that mitosis commences immediate-:ly after ovulation, before the luteal cells can well have become active, seems to eliminate this possibili-:ty. Also, the mitotic phase is of very short duration: it does not persist after about the sixth day of the cycle and mitotic activity during pregnancy is on a much lower level than during the post-oestrous phase. The mitotic phase bears about the same chronological relation to ovarian changes that is borne by the phase of desquamation in the vagina and the two may therefore have a common cause.

It has been remarked that the changes which take place in the cervix of the ewe during pregnancy and parturition are simply a gross exaggeration of those which take place during the dioestrous cycle. In pregnancy and in interoestrum tenacious mucus is actively secreted by the cervical epithelium: at parturition and in oestrum this mucus liquefies and flows into the vagina. These relations suggest that the secretion of mucus takes place under the influence of the luteal hormone and that liquefaction ensues with the degeneration of the corpus luteum.

The/

The formation of the miniature cervical plug during interoestrum could then be regarded as a "pseudo-pregnancy" change and the liquefaction of this mucus in oestrum as a pseudopregnant degeneration following atrophy of the corpus luteum. There is evidence to suggest, however, that the formation of mucus is not dependant upon the luteal hormone but more probably upon the follicular hormone and that the oestrous liquefaction is not strictly analogous to that of parturition. It has been seen that mucus is secret-:ed in small amounts during anoestrum and even in the immature animal, in which, presumably, no luteal hormone is present. Also, the greatest amount of secretory activity during the dioestrous cycle occurs during oestrum when the corpus luteum is atrophic. That the oestrous flow of mucus is not due solely to the atrophy of the corpus luteum is shown by the fact that the flow does not occur when the last corpus luteum formed during the breeding season atrophies. It is possible, naturally, that both the follicular and the luteal hormones may stimulate mucus secretion but it has been shown experimentally that "mucification" of the vagina in rodents during preg-:nancy is due, not to the luteal hormone but to the small amount of follicular hormone present (Robson (135), Robson and Wiesner (136), Meyer and Allen (113)). It is thus possible that the secretion of the/

the cervical mucus and the "secretory" modification of the vaginal mucosa of the ewe during pregnancy may be due to the presence of a small amount of oestrin. Turner et al (161) have shown that oestrin is produced during pregnancy in the cow and it is thus highly probable that it is produced in the ewe also. Liquefaction of the mucus plug of dioestrum is associated with increased secretory activity and is probably largely caused by the follicular hormone: at parturition there are no ripening follicles pres-:ent. Also, there is little reason to assume that liquefaction of the cervical mucus is directly brought about by either hormone: the effect is probably brought about simply by the vascular and other changes which accompany oestrum and parturition. Inflammation of the vagina or cervix due to infection is associated with a flow of mucus.

The immediate cause of liquefaction of the mucus plug is uncertain. It may depend upon an increase in alkalinity: against this view it may be stated that Zieger (quoted by Hammond) found that in the cow the cervical plug itself is strongly alkaline in reaction. However, Kessler and Uhr (81) found that there was an increase in acidity in the human vagina during pregnancy. Kurzrok and Miller (87, 88) have found that an enzyme contained in human seminal fluid exerts a lytic action upon the cervical/

cervical mucus. It is possible that lytic enzymes may be secreted by the ewe and cow under certain conditions.

The ewe and the cow appear to be remarkable, among the mammals that have been investigated, for the degree of development and complexity of their cervical passages and for the intensity of the secretory changes which take place in the cervical epithelium. Cervical mucus is by no means confined to these animals, however: it occurs in most, but the amount produced is much less proportionally and the cervical plug is especially characteristic of these two. The relation of mucus secretory changes in the cow to the ovarian changes appears to be almost identical with that in the ewe (66,39,118,119). A slight difference does exist however for in the cow (66), there is a short phase following oestrum when mucus is not secreted but is stored in the epithelial cells: this phase does not occur in the ewe.

In the cow, mucus-secretory cells are found in the vagina as well as in the cervix. Mucus cells occur also in the vaginae of many other forms under certain physiological conditions, e.g., in the Rodents during pregnancy. Mucus is secreted also by the uteri and oviducts of certain mammals. In the ewe mucous cells are confined at all times to the epithelia/

epithelia of the cervix and of the vagina in the immediate vicinity of the external os; but the "secretory" modification of the vaginal epithelium during pregnancy may be taken to indicate that this epithelium possesses mucus-secreting potentialities.

The distribution of mucus during pregnancy can leave little doubt as to its primary function. It would be difficult to devise a more adequate seal to the neck of the uterus than is provided by the cervical plug. The liquefaction of the plug must, naturally, take place before parturition begins but it is doubtful whether the function commonly ascribed to the mucus, of lubricating the vagina for the passage of the foetus, can be substantiated, for the mucus must necessarily be almost completely washed from the cervix and vagina by the escape of the foetal fluids. The foetus provides its own lubricant for the coat is always soaked with a thick, slimy substance.

The mucus flow of oestrum may, perhaps, serve to flush out the vagina and to provide a suitable medium for the sperm. It no doubt also assists coitus by "lubricating" the vagina and vulva. The formation of the rudimentary mucus plug during interoestrum may best be regarded as a "pseudopregnancy" change.

When/

When the vaginal changes in the ewe are compared with those in the Rodents (95, 75, 1, 16, 158, 146, 78) with the exception of the rabbit (85,147) the oestrous cycle in which is peculiar, it becomes obvious that the alterations taking place are entirely homologous : such differ-:ences as exist are differences in intensity and in chronological relation to the mating period. Mitosis in the rat's vagina is practically in abeyance during dioestrum and is intense during pro-oestrum and oestrum: in the sheep, the frequency of mitosis during dioestrum is comparable to that in the rat, but the increase in frequency which takes place at oestrum is very slight. Similarly, keratinisation, desquamation of the superficial epithelium and leucocytic infiltration are all subject to cyclical changes in the sheep as in the Rodents but the alterations are much less marked and (except in the case of desquamation) their precise relations to the oestrous cycle have been difficult to establish. That the most characteristic of the vaginal changes, viz., keratinisation and desquamation of the super-:ficial elithelium are due ultimately to the presence in the blood of the ovarian follicular hormone has been established experimentally in the Rodents and it is beyond reasonable doubt that the same cause is operative in the sheep. But whereas in the Rodents these/

these processes commence in pro-oestrum and are usually completed before the end of "heat", in the sheep they are not initiated until the latter half of heat and are not, as a rule, reflected in the vaginal smear until metoestrum. The dense "cornified" smear is characteristic of oestrum in the rodent but of metoestrum in the ewe. Since ovulation, which may probably be taken to mark the end of the period of oestrin secretion, takes place at about the same time in relation to the heat period in ewe and rat it may be concluded that the phase of oestrin secretion is also approximately coincident in the two forms. The reasonable explanation of these relations would appear to be that the ewe's vagina responds not only less intensely but much more slowly than the rat's to the stimuli created by the presence of oestrin in the blood stream. It has been shown (33) that the hormonic stimulus giving rise to the vaginal changes in the mouse is initiated about two days before the changes actually begin: injection of oestrin is effective after two days and ovariotomy 36 to 48 hours before oestrum is due to occur does not prevent the occurrence of the vaginal changes, so that it is not unreasonable to suppose that the duration of the lag differs in different animals. The alternative explanation, that the concentration of oestrin is lower in the sheep than

in/

in the rat and therefore acts more slowly and less intensely, requires the assumption that the mating desire is elicited by a much lower concentration of hormone in the ewe than in the rat, or that an enormous excess of oestrin is produced by the rat; and there is at present no evidence to suggest that either of these conditions exists. While superficial consideration suggests that desquamation might be due to the removal of oestrin, rather than to its actual presence, the suggestion does not bear close examination for undoubtedly in some few cases desquamation is initiated shortly before ovulation, at a time when the "oestrin" phase should be at its height. Continued injection of oestrin in Rodents causes protracted keratinisation and desquamation. Nymphomania in the cow is associated with prolongation of the type of vaginal smear characteristic of metoestrum, which is the phase of keratinisation and desquamation in the cow as in the sheep (57).

While the vaginal changes are ultimately referable to the presence of oestrin in the blood circulation, it may be doubted whether they are in any sense specific effects of this hormone. The change may be due simply to the increased blood supply to the reproductive organs during oestrus. If vaginal proliferation is a specific effect of oestrin,/

oestrin, there is no adequate explanation of why those changes do not occur synchronously in other epithelia of the same type, e.g., in the skin. Stieve (157) claims to have found "cyclical" changes in the oesophagus and mouth of the human female analagous with those in the vagina but since there is much dispute as to the existence of cyclical changes in the vagina of the woman this finding is not at present of great value. One of the pecularities of the sheep is that keratinisation is very localised. The whole epithelium including the stratum germinativum may be keratinised in some areas while in adjacent areas no keratinisation at all is to be seen. These differences could not be correlated with other structural differences, such as the distribution of blood capillaries, etc.

Desquamation is commonly looked upon as a secondary effect of keratinisation, but it has been shown, in the sheep and in other mammals, that the metoestrous desquamation is frequently not preceded by keratinisation. Nor can desquamation be regarded as due simply to the removal of the cells from the stratum germinativum for there is no oestrous wave of growth in the sheep's vagina. Giesbertz (63) believes that desquamation from the vagina of the woman is due to high glycogen content of the cells.

In comparison with the other domestic Ungulates/

Ungulates the vaginal changes in the ewe appear to be very distinctive. In the cow (Hammond (66), Murphey (118,119), Cole (39), Frei and Metzger (58), Schatalow (143)) there are apparently only slight changes in the epithelium: keratinisation and desquamation are poorly expressed. In the cow the vaginal epithelium is largely composed of mucus-secreting cells, so that it is in some way more strictly comparable to the cervix than to the vagina of the ewe. The phases of mucus secretion both in the vaginal and the cervical cells, correspond closely to those in the sheep. On the other hand leucocytosis is much more marked in the cow than in the sheep and vaginal haemorrhage, quite a common phenomenon in the cow, never occurs in the ewe, even sub-epithelial haemorrhage being absent.

Conditions in the sow do not appear to be very well known, although they have been studied (McKenzie (111), Zupp (171), Wilson (168)) but the indications are that changes in the vaginal epithelium of this animal are similar to, but much less definite than, those in the sheep. According to Wilson there is an oestrous proliferation and a metoestrous degeneration of the epithelium, unaccompanied by keratinisation. In the mare (Miller (115), Schatlow (142, 143)) changes in the vagina appear to be very slight and irregular. Conditions in the goat do not appear/

appear to have been studied but external manifesta-:tions of vaginal changes are very slight. The Ungulates are not peculiar in exhibiting slight vaginal changes: rather would it appear, from what little is known of other groups, that the Rodents are peculiar for the intensity of their cyclical vaginal phenomena.

The coincidence of keratinisation with the mating phase in Rodents has led to the view that keratinisation of the vagina is a protective measure against possible injury to the mucosa during coitus. In relation to the sheep, in which keratinisation is essentially a post-oestrous phenomenon, this inter-:pretation loses its significance. The writer is disposed to regard keratinisation as a purely second-:ary phenomenon without functional significance. In the sheep, were the keratinisation and desquamation phase to take place during oestrum, it would probably provide a barrier against effective coitus for the vaginal surface is so dry and sticky at this time that penetration by the male would be very difficult, if not entirely prevented. The mass of dry cellular material in the vagina would also absorb the sperm and thus further militate against insemina-:tion.

It has been seen that pregnancy is character-:ised by a type of vaginal mucosa not very different from/

from that found in dioestrum and anoestrum. It is interesting to note that pregnancy is the only phase of reproductive activity in which keratinised cells are never found. Since considerable amounts of oestrin are secreted during pregnancy in the cow (161) and probably therefore in the ewe, it might be expected that keratinisation would occur during pregnancy. The luteal hormone may perhaps inhibit the action of oestrin upon the vaginal mucosa.

The modifications of the accessory reproductive organs in the ewe are, as would be expected, virtually identical with those in the cow. The relations which these changes bear to the ovarian cycle are also the same in both animals. In the cow, however, heat seems to occur earlier than in the ewe so that early oestrous changes in the ewe tend to occur in late oestrum or early metoestrum in the cow. It seems therefore, that the lag in the mechanism responsible for the elicitation of the psychological manifestation of heat is greater in the ewe than in the cow.

Fertility.

While no direct study of the factors controlling fertility has been made during this investigation the subject is one of such outstanding importance in practical sheep husbandry that it may be/

be profitable to consider some of the possible bearings of this study upon breeding practice.

The "flushing" of ewes shortly before the breeding season is well known to be conducive to high fertility (Marshall (102,103,104,105), Nichols (120,121,122), Mohler (116), etc.). The effect of the extra feeding has been assumed to be due to stimulation of the reproductive mechanism, a greater number of ova being shed at the time of heat. So far as the author is aware, however, this view has never been confirmed by direct comparison of data derived from the examination of the ovaries of flushed and unflushed ewes.

It has been remarked that nutritive conditions appear to have little effect upon the rhythm of ovarian activity: the breeding season, the duration, periodicity and intensity of oestrum, and the intensity of the secondary phenomena of oestrum all appear to be little affected by nutritive conditions. It is a matter of some difficulty therefore, for the writer to accept the view that the number of Graafian follicles which mature and rupture at oestrum can be influenced profoundly by the rather temporary and superficial nutritive changes which, in practice, are constituted by "flushing". The writer is disposed to believe therefore that flushing may be operative through the conception rate rather than/

than through the level of ovarian function.

Hammond (64) examined the ovaries of pregnant ewes killed at abattoirs and obtained the following results. Eighty ewes showed a total of 116 corpora lutea in their ovaries; 101 normal foeti and 8 atrophic ones were found while seven of the corpora were unrepresented by foeti or foetal remains. Since the ewes were killed in February, March and April most of the ewes were presumably at least three months pregnant and some foeti may have been completely absorbed. From these results Hammond concluded that "the fertility of sheep is mainly influenced by those factors which control the number of eggs shed". This statement is manifestly entirely justified. Subsequent writers, however, have interpreted Hammond's statement as implying that "flushing" is effective through the number of ova shed from the ovaries, and this extension does not appear to the present writer to be entirely justifi-:able.

The following figures are quoted from Mohler's report of a controlled experiment to ascertain the effect of flushing upon fertility.

Ewes flushed upon extra good pasture	140 lambs per 100 ewes.
Ewes flushed with grain	136 lambs per 100 ewes.
Ewes unflushed	126 lambs per 100 ewes.

These figures may not be entirely representative/

representative but it may be stated as a generatisa-:tion that flushing causes a 25% to 33% increase in fertility. Returning to Hammond's figures it will be seen that foetal atrophy was responsible for the death of from 8 to 15 foeti in 80 ewes (according to the number of foeti which had completely atrophied) that is, for from 10 to 19 foeti per 100 ewes. The material examined probably came almost entirely from ewes which had been fattened for slaughter and most of them had probably been started on the fattening process by the time that they were served. In a sense therefore, these were flushed ewes. Since foetal atrophy was responsible for a 15% drop in the potential fertility of these ewes it does not appear to be unreasonable to suppose that early foetal atrophy involving one foetus of a multiple pregnancy may be solely responsible for the lower fertility of unflushed ewes as compared with those which receive special feeding at the time of service, and certainly the possibility that foetal atrophy is involved to some extent may not be eliminated.

It has been noted that the foetal membranes do not commence to become attached to the uterine mucosa until about the 26th to 27th day of pregnancy, and that until this happens the foetus is nourished almost entirely by the secretions of the uterine epithelium and glands. Moreover, from about the 16th to/

to the 25th day the membranes which are in organic connection with the foetus are not in contact with the uterine mucosa, since the completion of the amion separates the foetus entirely from the chorion. It seems probable therefore that this period is one of the most critical phases in the development of the foetus and one in which the foetus may be highly sensitive to deficiencies in maternal nutrition.

The writer is of the opinion therefore that an adequate level of maternal nutrition during the first month of pregnancy is one of the most necessary requirements for high fertility and that it has yet to be shown that "flushing" increases the number of ova shed during oestrum.

The correct resolution of this problem is clearly a matter of profound importance since, if it is the conception rate and not the level of ovarian activity that is affected by nutritive conditions, then "flushing" should be continued at least during the first month after service and many farmers are in the habit of withdrawing the extra food immediately all the ewes have been served.

VI : S U M M A R Y.

1. The age at puberty in the females of most domesticated breeds of sheep is 6 to 9 months and ewes are fertile at this age. There is evidence to suggest that certain of the more primitive breeds do not reach puberty until the second autumn after birth.

2. In the ewe, sterility does not result from increasing age so long as general bodily health is retained. It is suggested that the gradual decrease in fecundity during later life is due to declining bodily health.

3. A brief account is given of the growth and differentiation of the reproductive organs from birth to sexual maturity.

4. There is a well defined prepubertal phase of rapid growth in the uterus and ovaries. The uterine glands are formed during this phase.

5. The fundamental nature of the mammalian breeding season is emphasised and its evolutionary significance is discussed.

6. A survey of published data regarding the breeding season in domesticated sheep has been made.

7. The duration of the breeding season in Scottish sheep has been determined by direct observation of/

of a small flock and by the observation of ovarian activity in slaughtered ewes.

It was found that, in mature Scottish sheep generally, the breeding season lasts from about the middle of October until about the end of February and includes about 7 or 8 dioestrous cycles. Exceptionally, it may last for as much as 7 months and include 12 or more cycles.

8. In ewe lambs 6 to 9 months old the breeding season commences about a month later and ends about a month earlier than in mature animals.

9. The existence of breed differences affecting the duration of the breeding season is noted and evidence is produced to show that there are no considerable differences between the various Scottish breeds in this respect.

10. Minor differences in nutritive conditions do not affect the duration of the breeding season.

11. The average date of onset of the breeding season is subject to breed differences: most of the Scottish breeds do not differ greatly from one another in this respect but the Shetland breed comes into season fully a month later than the others.

12./

12. There is evidence to suggest that, in breed crosses, "early breeding" behaves as a dominant or incompletely dominant genetic character.

13. Nutritive "flushing" hastens the onset of the breeding season. It is suggested that an improvement in nutritional conditions converts phases of follicular maturation which would not otherwise be accompanied by the manifestation of heat into normal heat periods, and does not hasten follicular maturation (see also 32).

14. The effects of climate and environment upon the limits of the breeding season are discussed and the absence of comparative data noted.

15. The influence of seasonal changes in the environment upon the onset of the breeding season is discussed in relation to the cause of the restriction of the breeding season, and the tentative hypothesis is advanced that reproductive activity in the ewe is limited primarily by the diurnal duration and intensity of daylight, but that warm climates are conducive to high reproductive activity in the sheep as in other mammals.

16. The duration and intensity of light are thought to influence reproductive activity through the anterior/

anterior pituitary gland. Inhibition of pituitary activity by substances elaborated by photochemical reactions in the eye during an-:oestrum is suggested as a possible mechanism.

17. The results of an experiment designed to test the hypothesis that light is the principal factor in controlling reproductive activity in the ewe are presented and discussed. The results of this experiment were suggestive but inconclusive.

18. The conclusion of previous authors that wild sheep are monoestrous is criticised.

19. The durations of 63 normal dioestrous cycles and of 77 normal oestrous periods have been determin-:ed. The mean duration of the dioestrous cycle was 16.4 days (mean deviation $\pm$ 0.8 days) the modal duration was 16.5 days and the range of variation was from 15 to 18.5 days. The mean duration of oestrum was 36 hours (mean deviation $\pm$ 22 hours), the modal duration was 28 hours and the range of variation was from 3 to 84 hours.

20. The mean duration of the dioestrous cycle deduced from all available data and representing 697 cycles was 16.8 days, and the chief modal duration was 17 days. The range of variation was from 6 to 68 days but the distribution was multimodal.

21./

21. It is suggested that multiplication of the cycle duration takes place as a result of the occurrence of normal ovulation without the exhibition of the mating instinct (see also 32).

22. The influences of internal and external factors upon the dioestrous cycle are discussed. It is thought that breed, age, climate, environment and nutrition may all affect the duration and periodicity of oestrum slightly but conclusive evidence is lacking. Sterile service does not affect the cycle in any way.

23. Removal of the corpus luteum appears to cut short the dioestrous cycle but the evidence for this is slight.

24. Long heat periods are more intense than those which are of short duration.

25. There is no correlation between the duration of heat and the duration of the preceding or subsequent dioestrous cycle, but there is a negative correlation between the duration of heat and the duration of both preceding and subsequent interoestrous periods.

26. The end of heat is more labile than its commencement.

27./

27. It is postulated that the intensity and duration of heat are determined by the amount of heat-causing hormone released during the maturation of the follicle and that heat ceases when the amount of this hormone in the blood falls below a certain threshold value. The time of ovulation is not thought to affect the duration of heat unless it is definitely delayed.

28. The changes in the behaviour and in the external genital organs of the ewe during the dioestrous cycle are described.

29. It is concluded that it is possible to determine the limits of the heat period only by observing the mutual behaviour of the ram and ewe.

30. Heat comes on very rapidly and disappears slowly.

31. The gross changes in the ovaries during anoestrum and the dioestrous cycle are described. During anoestrum follicular maturation and rupture are in abeyance. Each dioestrous cycle is associat-:ed with the growth and rupture of a crop of Graafian follicles, with the development from the ruptured follicles of corpora lutea, and the subsequent atrophy of these.

32. Conclusive evidence is presented to show that normal maturation and rupture of Graafian follicles/

follicles and the development of corpora lutea occur prior to the commencement of the breeding season and occasionally during the breeding season without the exhibition of the mating instinct.

33. Interoestrum is associated with the development of corpora lutea. Full development of the corpora is attained by about the eighth day after the commencement of heat. Atrophy sets in on about the sixteenth day of the cycle and is soon followed by the onset of a new heat period.

34. Oestrum is associated with the very rapid increase in size of one or more Graafian follicles. These rupture about mid-oestrum.

35. Growth curves illustrating the development of the Graafian follicle and the development and atrophy of the corpus luteum during the dioestrous cycle are given.

36. The diameter of the follicle at the time of rupture varies considerably but it is usually about 10 millimetres.

37. The changes in the follicle immediately before rupture are described.

38. The literature on the subject of the cause of ovulation/

ovulation in mammals is discussed, and it is concluded that although the stimulus to follicular maturation and dehiscence probably originates from the pituitary gland, the immed-:iate cause of rupture is the accumulation of fluid, probably chiefly blood plasma, within the follicle.

39. Published data regarding the time of ovulation in the ewe are reviewed and it is concluded that ovulation in those breeds for which data are available takes place about 30 hours after the commencement of heat.

40. Reasons are given for the belief that ovulation occurs about 18 to 24 hours after the commence-:ment of heat in Scottish sheep.

41. Ovulation is entirely spontaneous and there is no evidence that it can be hastened by coitus.

42. The view is adopted that heat is caused by a hormonic stimulus emanating from the ovaries during the time when follicles are maturing, but that the hormone produced by the ovary does not itself stimulate the nervous system but is effective through a further hormonic link.

43. It is thought that the immediate regulator of oestrous periodicity is the corpus luteum and it is/

is postulated that the life of the corpus luteum is determined by the duration of secretion, probably by the pituitary, of some substance necessary for the life of the luteal cells, or perhaps by the rhythmic secretion of some substance inhibiting luteal growth.

44. The anatomy and histology of the uterus, cervix and vagina are described briefly, and a detailed account is given of the modifications in the structure of these organs associated with the dioestrous cycle and with anoestrum.

45. The uterus is slightly congested during oestrum and early metoestrum; slight extravasation of blood sometimes occurs in late oestrum or early metoestrum and there may be haemorrhage into the uterine lumen, but the amount of blood discharged is rarely sufficient to be detectable macroscopi-:cally. During anoestrum the uterus becomes anaemic.

46. The uterine stroma becomes oedematous during oestrum and early metoestrum.

47. During anoestrum the epithelium of the uterus and the uterine glands is reduced to cubical or low columnar form and the lumina of the glands are occluded. The amount of cytoplasm in the epithelial/

epithelial cells is greatly reduced and it is non-granular and non-vacuolated. There is no secretory activity.

48. Oestrum is marked by a phase of secretory and degenerative changes in the epithelia of the uterine surface and glands. The limits of this phase are subject to considerable variation.

49. During interoestrum these epithelia hypertrophy and secretory activity is in abeyance.

50. There is a phase of mitotic activity in the uterine epithelia lasting from about the time of ovulation until about the sixth day of the cycle. During dioestrum and early oestrum mitotic activity is in abeyance.

51. The stroma nuclei hypertrophy slightly during dioestrum. Mitotic division of the stroma nuclei is most common during the same phase of the cycle in which mitosis occurs in the epithelia, but is always rare.

52. Lymphocytes are present in the uterine mucosa during all phases of the dioestrous cycle and their number shows no cyclical variation. They are distributed throughout the mucosa and pass through the epithelia into the lumina of the uterus and uterine glands. Leucocytes are found in/

in the uterine mucosa only during pregnancy and the puerperium.

53. The uterine mucosa is usually deeply pigmented, especially on the cotyledons. The pigmentation is due to the presence of a subepithelial layer of melanoblasts. Pigmentation bears no relation to the stages of reproductive activity except that it largely disappears in pregnancy. It is found in prepubertal and in foetal animals. The melanin is identical with that in the skin. The Fallopian tubes are similarly pigmented.

54. There appear to be breed differences in the occurrence of uterine pigment but pigment is found in the uteri of all common Scottish breeds of sheep.

55. It is suggested that the pigment possesses no functional significance.

56. Other pigmented cells which are not dendritic and in which the pigment is non-granular are found during involution of the uterus. The pigment in these cells is probably of haematogenous origin.

57. The vagina, vestibule and vulva become congested and oedematous during early oestrum. Haemorrhage does not take place.

58./

58. Relaxation of the vaginal muscles occurs in oestrum.

59. There are no secretory cells in the vaginal epithelium.

60. Proliferation of the stratum germinativum of the vaginal epithelium is continuous during the dioestrous cycle but there is a slight increase in the frequency of mitosis during early oestrum. Mitoses are rare during anoestrum.

61. Intensive desquamation of the superficial layers of the vaginal epithelium takes place late in oestrum or early in metoestrum. At this time 4 or 5 layers of cells may be cast off. Slight desquamation takes place continuously throughout the dioestrous cycle and in anoestrum.

62. The thickness of the vaginal epithelium is greatest during oestrum, is reduced by the metoestrous desquamation and increases slowly through dioestrum. In anoestrum the epithelium is reduced below the metoestrous level.

63. Partial keratinisation of the superficial layers of the vaginal epithelium may occur at any time during the dioestrous cycle and in anoestrum but is essentially characteristic of late oestrum and metoestrum. Keratinisation is always regional.

64. Desquamation of the vaginal epithelium is not caused by keratinisation.

65. Lymphocytes are constantly present in the vaginal mucosa. They show no variation in number during the dioestrous cycle but are re-:duced in number during anoestrum.

66. Invasion of the vaginal mucosa and lumen by polymorphonuclear leucocytes may occur at any phase of the dioestrous cycle and in anoestrum but the tendency towards leucocytosis is greatest during dioestrum and least during anoestrum. Mechanical stimulation of the vaginal mucosa greatly increases the tendency towards leucocytic infiltration.

67. Changes in the character of the vaginal mucosa similar to those which occur during the dioestrous cycle take place during the last month of an-:oestrum in association with the cycles of follicular maturation and rupture which occur at that time, unaccompanied by the exhibition of "heat".

68. The glandulae vestibulares majores are vestigial and are thought to be devoid of functional significance.

69./

69. The muscles of the cervical canal relax during oestrum.

70. The epithelium of the cervical canal is composed entirely of mucus-secretory cells.

71. Secretion of mucus takes place continuously throughout the dioestrous cycle but is most intensive during oestrum. There is no secretion of mucus during anoestrum but slight amounts may be found in the epithelial cells.

72. The mucus secreted during interoestrum is tenacious and remains in the cervical canal, forming a rudimentary cervical plug.

73. During oestrum the cervical plug liquefies and the mucus flows into the vagina. The mucus secreted at this time is also fluid.

74. The liquefaction of the cervical mucus commences shortly before the onset of heat but the highest concentration of mucus in the vagina does not occur until heat is well advanced.

75. The value of the vaginal smear as a diagnostic measure in determining the stages of reproductive activity is discussed.

76. There are no gross changes in the mammary glands during the dioestrous cycle.

77./

77. A brief description of the changes in the reproductive organs during pregnancy is given and the changes in the uterine mucosa associated with the early development of the foetus and attachment of the foetal membranes are described in detail.

78. The development of the foetus is very slow until about the 15th day and thereafter very rapid until about the 27th day.

79. Attachment of the foetal membranes to the uterine mucosa commences to take place on about the 26th day after coitus: it does not commence until the allantois has become attached to the chorion and the uterus is distended by the expanding allantois. The embryo is in an advanced state of development before attachment begins.

80. Slight atrophy of the corpora lutea occurs on about the 15th day of pregnancy. They recover and become more strongly vascularised by the 18th day.

81. At about the 15th day secretory and degenerative changes commence to occur in the epithelium of the uterus and the epithelium of the uterine glands. Secretory activity continues throughout pregnancy in those parts of the surface epithelium which/

which are in contact with the foetal membranes but where the foetal membranes are not in contact with the mucosa the epithelium hypertrophies without secreting.

82. It is postulated that the presence of the foetus does not affect the normal dioestrous cycle until atrophy of the corpus luteum sets in. The secretory and degenerative changes in the uterine mucosa which then occur are thought to provide the foetus with the materials for rapid growth and the expansion of the foetal membranes is thought, by stimulating the uterine mucosa, to arrest the atrophy of the corpus luteum.

83. The epithelium covering the cotyledons is denuded by the phagocytic and digestive action of the foetal trophoblast. There is also slight erosion of the stroma and the surface capillaries break down.

84. The attachment of the foetal membranes is a reciprocal process in which both a burrowing action of the foetal trophoblast and hypertrophy of the stroma are involved.

85. The formation of the decidua is largely effected by hypertrophy of the stromal cells and by expansion/

expansion of the intercellular spaces but there is also proliferation of the stroma.

86. Modification of the cotyledons does not take place unless the mucosa is in contact with the allantochorion. Contact with the trophoblast alone is insufficient to induce change in the stroma.

87. The placental barrier is of the syndesmochorial type.

88. The uterine glands secrete intensively throughout pregnancy and become greatly dilated.

89. Until the 26th day the foetus is entirely dependant upon the secretions of the uterine epithelium and glands for its nourishment and it is postulated that these secretions constitute the principal source of nourishment of the foetus throughout pregnancy.

90. Atrophy of the corpora lutea of pregnancy commences at about the 18th week and the corpora are markedly atretic at the time of parturition.

91. Follicular maturation is completely in abeyance during pregnancy and the follicles which are present at the time of conception atrophy.

Atrophy of the corpora lutea is followed by follicular/

follicular growth but maturation and rupture of follicles do not occur until the following autumn.

92. It is concluded that follicular growth is inhibited by a secretion of the corpus luteum.

93. During pregnancy the cervical canal becomes distended by the accumulation within its lumen of mucus secreted by its epithelium. Secretion of mucus continues throughout pregnancy.

94. At parturition the mucus plug liquefies and great quantities of mucus flow into the vagina.

95. During pregnancy the superficial layers of the vaginal epithelium tend to become converted into cubical or columnar cells resembling in some ways the mucus secretory cells of the cervical epithelium, but mucus is never formed.

96. Desquamation ceases and keratinised cells are never found in the vagina during the later stages of pregnancy. Leucocytic infiltration is reduced to the anoestrous level.

97. The changes in the uterus, cervix and vagina during the dioestrous cycle are compared with those which occur during pregnancy: the functional significance and the relations of the changes/

changes to ovarian secretions are discussed. The changes are compared also, to those which have been described in other mammals.

98. The hypertrophic changes in the epithelia of the uterine mucosa during interoestrum are regarded as analogous to those which occur during pregnancy and are throught to be due to the presence of a luteal hormone. They are thus considered to be equivalent in physiological significance to those which occur in the rodents during "pseudo-:pregnancy".

99. The degenerative changes occurring in the uterus during oestrum are considered to be equivalent to both the pro-oestrous and the pseudopregnant degenerative phases in the bitch, the two being superimposed in the ewe by the rapid onset of heat following degeneration of the corpora lutea of the previous cycle.

100. The rudimentary mucus plug formed during inter-:oestrum is regarded as the physiological analogue of the true mucus plug formed during pregnancy. The secretion of mucus is thought to occur under the influence of the ovarian follicu-:lar hormone.

101. The liquefaction of the cervical mucus which occurs/

occurs during oestrum is not regarded as equivalent to that which occurs in parturition. Liquefaction is thought to be a non-specific effect due, probably, to vascular congestion of the cervix.

102. The primary function of the cervical mucus is thought to be the sealing of the cervix during pregnancy.

103. Keratinisation and desquamation of the vaginal epithelium are thought to be remote effects of the ovarian follicular hormone and to be devoid of functional significance.

104. Some possible bearings of this study upon the problem of the factors controlling fertility in the ewe are discussed. It is suggested that increased fertility as a result of nutritive "flushing" may be due to the prevention of death and resorption before attachment of the membranes to the uterine mucosa has taken place, of some of the foeti in multiple pregnancies; and that "flushing" may not influence the number of ova shed during oestrum.

A C K N O W L E D G M E N T S .

The author wishes to express his gratitude to Professor F.A.E. Crew, not only for the provision of research facilities but also for the deep interest he has displayed in the progress of this study. The work has come more directly under the supervision of Mr. Wm.C. Miller, Head of the Sheep and Wool Section of the Institute of Animal Genetics and to him the writer is deeply indebted for constant guidance and the benefits of his wide knowledge of all that pertains to the domesticated animals and to sheep especially. To Mr. Miller also, the writer is in-:debted for the performance of the major surgical operations which were involved. Miss M.V. Cytovich of the Imperial Bureau of Animal Genetics kindly undertook to translate literature in the Russian and Italian languages to which it was desired to make reference.

To the Authorities at the Edinburgh abattoir the writer is indebted for the provision of facilities for the collection of material. Without their co-operation and that of the killers, no adequate study of the subject could have been made.

B I B L I O G R A P H Y.

1. ALLEN, E.
"The oestrous cycle in the mouse."
1922: Amer. Jour. Anat., 30, p. 297.

2. ALLEN, E., F.F. McKENZIE, J.W. KENNEDY and W.K. BEARE.
"Oestrous periods, ovarian changes and tubal ova in the sheep."
1931: Anat. Rec., 48, Suppl. Art. 9. (Abst.).

3. ALLEN, W.M.
"I. Cyclical alterations of the endometrium of the rat during the normal cycle, pseudopregnancy and pregnancy. II. Production of deciduomata during pregnancy."
1931: Anat. Rec., 48, p. 65.

4. ALONGI, G.
"Sulla comparsa dei 'calore' e sulla loro durata in alcuni animali domestici (cavella, vacca, pecora, cagna, scrofa, capra) di varie regioni d'Italia."
1924: Rass. Studi Sess. Eugen., 4, pp. 85-91, 158-165.

5. AMIRTHALINGAM, C.
"On lunar periodicity in reproduction in repro-:duction in Pecten opercularis near Plymouth in 1927-28."
1928: Jour. Mar. Biol. Ass. (Plymouth), 15, pp. 605-641.

6. ANDERSON, J.
In preparation.

7. ANONYMOUS.
"A practical treatise on the Merino and Anglo-Merino breeds of sheep.... by an experienced breeder."
1809: London. W. Plant Piercy.

8. ANONYMOUS.
"Nutrition in relation to reproduction with special reference to sterility in farm animals." (Review)
1932: Imperial Bureau of Animal Nutrition, Rowett Institute, Aberdeen.

9./

9.*ASDELL, S.A.
"Thesis for Ph.D., Cambridge."
Cit. Hill and Parkes (72).

10. ASDELL, S.A.
"Variation in the onset of the breeding year in the goat."
1926: Jour. Agric. Sci., 16, pp. 632-639.

11. ASSHETON, R.
"The morphology of the Ungulate placenta, particularly the development of that organ in the sheep, and notes upon the placenta in the elephant and Hyrax."
1906: Phil. Trans. Roy. Soc. Lond. B, 198.

12. BAKER, J.R. and R.M. RANSON.
"Factors affecting the breeding of the field mouse (Microtus hirtus) - (1) Light."
1932: Proc. Roy. Soc. Lond. B, 110, pp. 313-322.

13.*BEARD.
"The span of gestation and the cause of birth."
1897: Jena. Cit. Marshall (101).

14. BELL, A.G.
"Sheep breeding experiments on Beinn Bhreagh."
1912: Science, N.S., 36, pp. 378-384.

15. BELLERBY, C.W.
"The relation of the anterior lobe of the pituitary to ovulation."
1929: Jour. Physiol., 67, pp. xxxiii - xxxiv.

16. BENAZZI, M.
"Osservazioni sulla struttura dell'epithelio vaginale di Mus musculus in alcune condizioni biologiche."
1930: Monit. Zool. Ital., 41, pp. 143-150.

17. BENAZZI, M.
"Contributo alla istofisiologia dell'utero di mammiferi con particulare riguardo alla conoscenza delle cellule pigmente."
1930: Arch. Ital. Anat. e Embriol., 28, pp. 163-210.

18./

18.* BENAZZI, M.
"Sull'origine del pigmento nell'utero di Mus musculus."
1930: Boll. Soc. Ital. Biol. Sper., 5, pp. 336-338.

19. BENEDICT, G.F. and E. RITZMAN.
"Uber die den Energieumsatz bei Schaffen beeinflussenden Faktoren."
1931: Arch. f. Tiernährung u. Tierzücht., 5, S. 1-88.

20. BISSONNETTE, T.H.
"Studies on the sexual cycle in birds. I: Sexual maturity, its modifications and possible control in the European starling (Sturnus vulgaris)."
1930: Amer. Jour. Anat., 45, pp. 289-305.

21. BISSONNETTE, T.H.
"Studies on the sexual cycle in birds. V: Effects of white light of different intensities upon the testis activities of the European starling (Sturnus vulgaris)."
1931: Physiol. Zool., 4, pp. 542-574.

22. BISSONNETTE, T.H.
"Studies on the sexual cycle in birds. VI: The effects of white, red and green lights of different intensities on testis activity of the European starling (Sturnus vulgaris)."
1932: Physiol. Zool., 5, pp. 92-123.

23. BISSONNETTE, T.H.
"Modifications of mammalian sexual cycles: reactions of ferrets (Putorius vulgaris) of both sexes to electric light added after dark in November and December."
1932: Proc. Roy. Soc. Lond. B, 110, pp. 322-336.

24. BISSONNETTE, T.H.
"Light and diet as factors in relation to sexual periodicity."
1932: Nature, 129, p. 612. (Letter to).

25./

25. BISSONNETTE, T.H. and M.H. CHAPNICK.
"Studies on the sexual cycle in birds. II: The normal progressive changes in the testis from November to May in the European starling (Sturnus vulgaris), an introduced, non-migratory bird."
1930: Amer. Jour. Anat., 45, pp. 307-343.

26. BISSONNETTE, T.H. and A.P.R. WADLUND.
"Spermatogenesis in Sturnus vulgaris: refractory period and acceleration in relation to wave length and rate of increase of light ration."
1931: Jour. Morph. Physiol., 52, pp. 403-428.

27. BONNET, R.
"Ueber Melanose der Uterineschleimhaut bei Schafen - I."
1880: Dtsch. Zeit. f. Tiermed., 6, S. 419.

28. BONNET, R.
"Zur Kenntniss der Uterinmilch."
1880: Dtsch. Zeit. f. Tiermed., 6.

29. BONNET, R.
"Ueber Melanose der Uterineschleimhaut bei Schafen - II."
1882: Dtsch. Zeit. f. Tiermed., 7, S. 365.

30. BONNET, R.
"Eigenthumliche Stäbchen in der Uterinmilch des Schafes."
1882: Dtsch. Zeit. f. Tiermed, 7.

31. BONNET, R.
"Ueber Embryotrophe."
1899: Dtsch. Med. Wschr., No. 45.

32. BOWSTEAD, J.E.
"The effect of breeding immature ewes."
1930: Sci. Agric., 10, pp. 429-459.

33. BRAMBELL, F.W.R. and A.S. PARKES.
"The normal ovarian cycle in relation to oestrous production."
1927: Quart. Jour. Exp. Physiol., 18, pp. 185-198.

34./

34. BRAMBELL, F.W.R. and A.S. PARKES.
"Studies on ovulation. VI: Relative importance of concentration and absolute amount of ovulation-producing hormone."
1932: Jour. Physiol., 74, pp. 173-178.

35. BRAMBELL, F.W.R., A.S. PARKES and U. FIELDING.
"Changes in the ovary of the mouse following exposure to X-rays. I: Irradiation at three weeks old."
1927: Proc. Roy. Soc. Lond. B., 101, pp. 29-56.

36. BRAMBELL, F.W.R., A.S. PARKES and U. FIELDING.
"Changes in the ovary of the mouse following exposure to X-rays. II: Irradiation at or before birth."
1927: Proc. Roy. Soc. Lond.B., 101, pp. 95-114.

37. CASIDA, L.E. and F.F. McKENZIE.
"The oestrous cycle of the ewe; histology of the genital tract."
1932: Univ. Missouri Agric. Expt. Sta. Res. Bull., 170.

38. COFFEY, W.C.
"Productive sheep husbandry."
1918: Philadelphia and London: Lippincott Co.

39. COLE, H.H.
"A study of the mucosa of the genital tract of the cow, with special reference to the cyclic changes."
1930: Amer. Jour. Anat., 46, pp. 261-302.

40. COLE, H.H. and R.F. MILLER.
"The vaginal smear of the ewe."
1931: Proc. Soc. Exp. Biol. Med., 28, p. 841.

41. COLE, H.H. and R.F. MILLER.
"Data on reproduction in the sheep."
1932: Anat. Rec., 52, Suppl. p. 49, (Abst.).

42. COLE, H.H. and R.F. MILLER.
"The vaginal smear of the ewe."
1932: Anat. Rec., 52, Suppl. p. 50, (Abst.).

43./

43. COLEBATCH, W.J. and R.C. SCOTT.
"An investigation into aspects of fat lamb production on agricultural holdings."
1928: Jour. Agric. South Australia.
(Reprint seen - no vol. given.)

44. CORNER, G.W.
"Cyclic changes in the ovaries and uterus of the sow and their relations to the mechanism of implantation."
1921: Carnegie Inst. Washington. Contrib. to Embryology, 13, pp. 119-146.

45. COURRIER, R. and R. RAYNAUD.
"Modifications génitales chez les animaux à croissance arrêtée par la déficience en lysine."
1932: C.R. Soc. Biol. Paris, 109, pp. 881-883.

46. CUNNINGHAM, J.T.
"Light as a factor in sexual periodicity!"
1932: Nature, 129, pp. 543-544. (Letter to).

47. DARLOW, A.E. and L.E. HAWKINS.
"The oestrous cycle in the sheep."
1932: Proc. Amer. Soc. Anim. Prod., Nov. 1931, pp. 205-207.

48. DARLOW, A.E. and L.E. HAWKINS.
"The influence of nutrition on the oestrous cycle in the ewe."
1933: Proc. Amer. Soc. Anim. Prod., Nov. 1932, pp. 173-176.

49. DAVIS, K.B.
"Periodicity of sexual desire. Pt. II. Married women."
1927: Amer. Jour. Obstet. & Gynecol., 14, pp.345-360.

50. DRIPS, D.G.
"Irregularity in the estrual cycle of the white rat following operation on the ovaries."
1929: Arch. Path., 8, pp. 187-199.

51. ECKLES, C.H., R.B. BECKER and L.S. PALMER.
"A mineral deficiency in the rations of cattle."
1926: Minnesota Sta. Bull., No. 229, pp. 4-49.

52./

52. ELLENBERGER, W. and H. BAUM.
"Handbuch der vergleichenden Anatomie der Haustiere." 16 Auflage.
1926: Berlin. Julius Springer.

53. EVANS, H.M. and O. SWEZY.
"Ovogenesis and the normal follicular cycle in adult mammalia."
1929: Mem. Univ. California, 9, pp. 119-224.

54. FEE, A.R. and A.S. PARKES.
"Studies on ovulation. I. The relation of the anterior pituitary body to ovulation in the rabbit."
1929: Jour. Physiol., 67, pp. 383-388.

55. FEE, A.R. and A.S. PARKES.
"Studies on ovulation. III. Effect of vaginal anaesthesia on ovulation in the rabbit."
1930: Jour. Physiol., 70, pp. 385-388.

56. FREI, W.
"Prophylaxis and therapy of sterility in the cow."
1930: Proc. 11th Internat. Vet. Congress (London).

57. FREI, W. and E. LUTZ.
"Der heutige Stand der Forschungen über das Oestrushormon und die Nymphomania des Rhindes."
1929: Virchow's Arch. Path. Anat. u. Physiol., 271, S. 572-622.

58. FREI, W. and E. METZGER.
"Die Sexualperiodizität in der Vagina des Rindes."
1926: Berl. Tierarz. Wochenschr., 39, S. 645.

59. FRIEDMAN, M.H.
"The mechanism of ovulation in the rabbit. I. Demonstration of a humoral mechanism."
1929: Amer. Jour. Physiol., 89, pp. 438-442.

60. FRIEDMAN, M.H.
"The mechanism of ovulation in the rabbit. II. Ovulation produced by the injection of urine from pregnant women."
1929: Amer. Jour. Physiol., 90, pp. 617-622.

61./

61.* GAMGEE, A.
"On the chemistry and physiology of the milky fluid found in the placental cotyledons of ruminants."
1864: Brit. & Foreign Med. Chir. Rev., 33, p. 180.

62. GENTHER, I.T.
"Irradiation of the ovaries of guinea-pigs and its effects on the oestrous cycle."
1931: Amer. Jour. Anat., 48, pp. 99-137.

63. GIESBERTZ, H.
"Zur Frage der periodischen Veranderungen des menschlichen Schleimhautephithels."
1929: Arch. f. Gynakol., 136, S. 362-376.

64. HAMMOND, J.
"Further observations on the factors controlling fertility and foetal atrophy."
1921: Jour. Agric. Sci., 11, pp. 337-366.

65. HAMMOND, J.
"Reproduction in the rabbit."
1925: Edinburgh, Oliver and Boyd.

66. HAMMOND, J.
"The physiology of reproduction in the cow."
1927: Cambridge, University Press.

67. HAMMOND, J. and F.H.A. MARSHALL.
"Oestrous and pseudopregnancy in the ferret."
1930: Proc. Roy. Soc. Lond. B, 105, pp. 607-630.

68. HART, G.H.
"The scientific principle of nutrition with special reference to percentage calf crop in range herds."
1930: Proc. 11th Internat. Vet. Congress (London).

69. HARTMAN, C.G. and J. BALL.
"On the almost instantaneous transport of spermatozoa through the cervix and the uterus in the rat."
1930: Proc. Soc. Exp. Biol. Med., 28, pp. 312-314.

70./

70. HARTMAN, C.G., W.M. FIROR and E.M.K. GEILING.
"Menstruation and the anterior pituitary."
1930: Proc. Soc. Exp. Biol. Med., 28, p. 185.

71. HEAPE, W.
"The 'sexual season' of mammals and the relation of the 'pro-oestrum' to menstruation."
1900: Quart. Jour. Micr. Sci., 44, pp. 1-70.

72. HILL, M. and A.S. PARKES.
"The relation between the anterior pituitary body and the gonads. Pt. I. The factors concerned in the formation of the corpus luteum."
1930: Proc. Roy. Soc. Lond. B, 107, pp. 30-38.

73. HORLACHER, L.J.
"Sheep production."
1927: New York and London. McGraw-Hill Book Coy.

74. HUMPHREY, R.R.
"Effects of vasoligation in sheep and goats."
1926: Anat. Rec., 33, pp. 41-46.

75. ISHII, O.
"The sexual cycle of the white rat."
1922: Anat. Rec., 23, p. 311.

76. IVANOW, E.I.
"The relation of ovulation to heat in the sheep."
(In Russian.)
(Separate reprint in possession of Professor Crew: no Journal given. Date of publication about 1914.)

77. JONES, S.V.H. and J.E. ROUSE.
"The relation of age of dam to observed fecundity in domesticated animals. I. Multiple births in cattle and sheep."
1920: Jour. Dairy Sci., 3, pp. 260-290.

78. KAZZANDER.
"Uber die Pigmentation der Uterinschleimhaut des Schafes."
1890: Arch. f. Mikr. Anat., 36.

79./

79. KELLY, G.L.
"The histological transformations in the vaginal epithelium of the guinea pig."
1929: Amer. Jour. Anat., 43, pp. 247-288.

80. KENNEDY, W.P.
"Diet and sterility."
1926: Physiol. Rev., 6, pp. 485-503.

81. KESSLER, R. and E. UHR.
"Biologie und Chemismus der Scheide bei Schwangeren."
1926-27: Arch. f. Gynakol., 129, S. 844-855.

82. KOLLER, P.C.
"Experimental studies on pigment formation. I. The development in vitro of the mesodermal pigment cells of the fowl."
1929: Arch. f. Exp. Zellforschung., 8, pp. 490-498.

83. KOLSTER.
"Uber die Embryotrophe placentarer Sauger mit besonderer Beruchsichtigung der Stute."
1901: Anat. Hefte, Abt. 1, 18, S. 457.

84. KOLSTER.
"Weitere Beiträge zur Kentniss der Embryotrophe bei Indeciduaten."
1902: Anat. Hefte, Abt. 1, 20, S. 233.

85. KUNDE, M.M. and T. PROUD.
"The ineffectiveness of vaginal smears in predicting the oestrous cycle in the rabbit."
1929: Amer. Jour. Physiol., 88, pp. 446-452.

86. KÜPFER, M.
"The sexual cycle of female domesticated animals. The ovarian changes and the periodicity of oestrum in cattle, sheep, goats, pigs and horses (Observations on animals in Central Europe and South Africa.)."
1929: 13th and 14th Reports, Dir. of Vet. Educ. and Res. Dept. of Agric., Pretoria, U. of S.A., Pt. II, pp. 1209-1270, and Supplement.

87./

87. KURZROK, R. and E.C. MILLER.
"Mucin of the cervix uteri."
1927: Proc. Soc. Exp. Biol. Med., 24, p. 670.

88. KURZROK, R. and E.C. MILLER.
"Biochemical studies of human semen and its relations to the mucus of the cervix uteri."
1928: Amer. Jour. Obstet. & Gynecol., 15, pp. 56-72.

89.*KÜST.
"Beitrag zur Kenntniss der Rachitis und der Brullerkrankeit des Rindes."
1930: Tierarztl. Runds., 36, pp. 741-743, 766-769.

90.*LATASE.
1893: C.R. Soc. Biol. Mem., 45, p. 135.
Cit. Hammond (66)

91. LEE, M.C.
"Studies on the oestrous cycle in the rat. III. Effect of low environmental temperatures."
1926: Amer. Jour. Physiol., 78, pp. 246-253.

92. LLEWELLYN, Ll. J.
"Light and sexual periodicity."
1932: Nature (Letter to), 129, p. 868.

93. LOEB, L.
"The correlation between cyclical changes in the uterus and ovaries in the guinea-pig."
1914: Biol. Bull., 27, pp. 1-44.

94. LOEB, L.
"The experimental production of hypotypical ovaries through underfeeding."
1917: Biol. Bull., 33.

95. LONG, J.A. and H.M. EVANS.
"The oestrous cycle in the rat and its associated phenomena."
1922: Memoirs of the Univ. of California, 6.

96. LYDEKKER.
"The sheep and its cousins."
1912: London: George Allen & Co.

97. LYONS, W.R.
"Ovarian follicular fluid - a plasma transudate."
1932: Anat. Rec., 52, Suppl. p. 66, (Abst.).

98. MALDEN, W.J.
"British sheep and shepherding."
London: Macdonald and Martin.

99. MARRIAN, C.F. and A.S. PARKES.
"The effect of anterior pituitary preparations administered during dietary anoestrus."
1929: Proc. Roy. Soc. Lond. B, 105, pp. 248-258.

100. MARRIAN , G.F. and A.S. PARKES.
"The relative amounts of oestrin required to produce the various phenomena of oestrus."
1930: Jour. Physiol., 69, pp. 372-376.

101. MARSHALL, F.H.A.
"The oestrous cycle and the formation of the corpus luteum in the sheep."
1903: Philos. Trans. Roy. Soc. Lond. B, 196, pp. 47-97.

102. MARSHALL, F.H.A.
"Fertility in sheep."
1904: Trans. High. & Agric. Soc. Scotland, pp. 34-43.

103. MARSHALL, F.H.A.
"Fertility in Scottish sheep."
1905. Proc. Roy. Soc. Lond. B, 77, pp. 58-62.

104. MARSHALL, F.H.A.
"Fertility in Scottish sheep."
1908: Trans. High. & Agric. Soc. Scotland, pp. 131-151.

105. MARSHALL, F.H.A.
"The effect of environment and nutrition upon Fertility."
1908: Sci. Progress, 7, pp. 1-9.

106. MARSHALL, F.H.A.
" The physiology of reproduction."
1922: London: Longmans, Green and Co.

107./

107. MARSHALL, F.H.A. and J. HAMMOND.
"The physiology of animal breeding with special reference to the problem of fertility."
1931: Min. Agric. & Fish. Res. Monograph, No. 2, 3rd Ed.

108.* MAYOR, A.G.
"The annual breeding swarm of the Atlantic Palolo."
Carnegie Inst. Publ. 102.

109.* MAYO-SMITH.
"Statistics and Sociology." Vol. 1.
1895: New York. Cit. Marshall (106).

110. McCLUNG, C.E.
"Handbook of microscopical technique."
1929: New York, Hoeber Inc.

111. McKENZIE, F.F.
"The normal oestrous cycle in the sow."
1926: Univ. of Missouri Coll. of Agric. Res. Bull., 86.

112. McKENZIE, F.F. and R.W. PHILLIPS.
"Some observations on the estrual cycle in the sheep."
1931: Proc. Amer. Soc. Anim. Prod., Nov. 1930, pp. 138-143.

113. MEYER, R.K. and W.M. ALLEN.
"The production of mucification of the vaginal epithelium of rodents by the oestrous hormone."
1932: Science, 75, pp. 111-112.

114. MILLER, Wm. C.
Personal communication.

115. MILLER, Wm. C.
Personal communication.

116. MOHLER, J.R.
"Report of the Chief of the Bureau of Animal Industry." (United States).
1932: Aug.

117./

117.* MOREAU, E.R.
Ibis (13), 1, pp. 553-570.
Cit. Bissonnette (24).

118. MURPHEY, H.S.
"Studies of the oestrous cycle in the ox."
1922: Anat. Rec., 23, p. 29, (Abst.)

119. MURPHEY, H.S.
"Studies of the oestrous cycle in the ox."
1926: Iowa State Coll. Vet. Pract. Bull., 25, pp. 153-163.

120. NICHOLS, J.E.
"Fertility in sheep."
1924: Jour. Min. Agric., 31, No.9.

121. NICHOLS, J.E.
"Fertility in sheep."
1926: Jour. Min. Agric., 33, No. 3.

122. NICHOLS, J.E.
"Some observations on the problem of fertility and fecundity in sheep."
1927: Zeit.f. Tierzüchtung u. Züchtungsbiol., 10, S. 226-234.

123. PAPANICOLAOU, G.N. and C.R. STOCKARD.
"Effect of under feeding on ovulation and the oestrous rhythm in guinea pigs."
1920: Proc. Soc. Exp. Biol. Med., 17, p. 143.

124. PARKES, A.S.
"On the occurrence of the oestrous cycle after X-ray sterilisation. I: Irradiation of mice at three weeks old."
1926: Proc. Roy. Soc. Lond.B., 100, pp. 172-199.

125. PARKES, A.S.
"On the occurrence of the oestrous cycle after X-ray sterilisation. II: Irradiation at or before birth."
1927: Proc. Roy. Soc. Lond. B., 101, pp. 71-95.

126./

126. PARKES, A.S.
"On the occurrence of the oestrous cycle after X-ray sterilisation. III: The periodicity of oestrus after sterilisation of the adult."
1927: Proc. Roy. Soc. Lond. B, 101, pp. 421-449.

127. PARKES, A.S.
"The nature of the anoestrous condition result-:ing from vitamine B deficiency."
1928: Quart. Jour. Exp. Physiol., 18, pp. 397-401.

128. PARKES, A.S., I.W. ROWLANDS, and F.W.R. BRAMBELL.
"Effect of X-ray sterilisation on oestrus in the ferret."
1932: Proc. Roy. Soc. Lond. B, 109, pp. 425-434.

129. PEARL, R.
"Note regarding the relation of age to fecundity."
1913: Science, N.S., 37, pp. 226-228.

130. QUINLAN, J. and G.S. MARÉ.
"The physiological changes in the ovary of the Merino sheep in South Africa, and their practical application in breeding."
1931: 17th Rept. of the Dir. of Vet. Services & Animal Indust., U. of S.A., pp. 663-701.

131. REISS, M. and PÉRENY.
"Der Einfluss der Futterung mit poliertem Reis auf die Brunstreaktion der Ratte."
1928: Endokrinologie, 1, S. 411-418.

132. RETTERER, E.
"Evolution de l'épithélium du vagin (Deuxième note)."
1892: Mem. C.R. Soc. Biol. Paris, 44, pp. 566-568.

133. RICHTER, J.
"Ursachen und Behandlung der Unfructbarkeit des Rindes."
1922: Berlin: Schoetz.

134. ROBINSON, A.
"The formation, rupture and closure of ovarian follicles in ferrets and ferret-polecat hybrids and some associated phenomena."
1918: Trans. Roy. Soc. Edin., 52, Pt. II, p. 303.

135. ROBSON, J.M.
"Mucification in the mature mouse caused by oestrin."
1930: Jour. Physiol., 71, p. iii.

136. ROBSON, J.M. and B.P. WIESNER.
"Causation of mucification and cornification in the vagina of the mouse."
1931: Quart. Jour. Exp. Physiol., 21, pp. 217-225.

137.*ROWAN, W.
"On photoperiodism, reproductive periodicity and the annual migrations of birds and certain fishes."
1926: Proc. Boston. Soc. Nat. Hist., 38, pp. 147-189.

138. ROWAN, W.
"Migration and reproductive rhythm in birds."
1927: Nature, 119, p. 351, (Letter to).

139. ROWAN, W.
"Reproductive rhythm in birds."
1928: Nature, 122, pp. 11-12.

140. SAKURAI, T.
"Normentafel zur Entwicklungsgeschichte des Rehes (Cervus capreolus)."
1906.

141. SANCTIS, A. de
"Periodi di calore o di frega in alcuni mammiferi."
1926: Rass. Studi Sess. e Eugen., 6, pp. 10-18.

142. SCHATALOW, P.
"Zur Frage der Sexualzyklen." (In Russian).
1930: Vetinärzootechnischer Bote No. 2.

143. SCHATALOW, P.
"Zur Frage des Geschlechtszyklus der Pferde und Kühe nach dem mikroskopischen Bilde des Vaginal Austriches."
1933: Berlin Tierartz. Woch., 49, S. 81-84.

144./

144. SCHMALTZ, R.
"Das Geschlechtsleben der Haussäugetiere."
1921: Berlin: Schoetz. - 3 Auflage.

145.* SCHOCHET.
"A suggestion as to the process of ovulation and ovarian cyst formation."
1916: Anat. Rec., 10. Cit. Hammond (66).

146. SELLE, K.M.
"Changes in the vaginal epithelium of the guinea pig during the oestrous cycle."
1922: Amer. Jour. Anat., 30, pp. 429-449.

147.* SHIBATA, S.
"The oestrous cycle and ovulation in the rabbit."
1931: Jour. Coll. Agric. Imp. Univ. Tokyo, 11, pp. 309-340.

148.* SHIRAKI, K.
"Study on the relation of the fat to the sexual cycle."
1929. Trans. Jap. Path. Soc., 19, pp. 83-84.

149. SLONAKER, J.R.
"The effect of different percentages of protein in the diet. IV: Reproduction."
1931: Amer. Jour. Physiol., 97, pp. 322-328.

150. SLONAKER, J.R. and T.A. CARD.
"The effect of a restricted diet. II: On pubescence and the menopause."
1923: Amer. Jour. Physiol., 64, pp. 35-43.

151. SLONAKER, J.R. and T.A. CARD.
"The effect of a restricted diet. III: On the number of litters and young born."
1923: Amer. Jour. Physiol., 64, pp. 167-180.

152. SLONAKER, J.R. and T.A. CARD.
"The effect of a restricted diet. IV: On the age of greatest productivity."
1923: Amer. Jour. Physiol., 64, pp. 203-209.

153./

153. SMITH, A.D.B.
"A twenty-one-year-old ewe."
1927: Jour. Hered., 18, No.4.

154. SMITH, A.D.B.
"Superfoetation - further cases in pigs and sheep."
1927: Jour. Anat., 62, pp. 100-104.

155. SMITH, F.
"Veterinary Physiology."
1921: London; Bailliere, Tindall and Cox.
5th Ed.

156. SNYDER, F.F. and G.B. WISLOCKI.
"Further observations upon the experimental production of ovulation in the rabbit."
1931: Bull. Johns Hopkins Hospital, 48, p. 106.

157. STIEVE, H.
"Verhornungserscheinungen im Epithel der menschlichen Speisröhren und Scheidenschleimhaut."
1931: Zeit. mikrosk.-anat. Forschg., 24, S. 213-236.

158. STOCKARD, C.R. and G.N. PAPANICOLAOU.
"The existence of a typical oestrous cycle in the guinea pig: with a study of its histological and physiological changes."
1917: Amer. Jour. Anat., 22, pp. 225-283.

159. THILO, H.L.
"Die Zucht des Meleschafes in Neuenkirchen."
1922: Privately printed by V. Ziemsen, Halle.

160. TRAUTMANN.
"Anatomisches und Histologisches über die Cervix uteri. I Mitteilung."
1917: Arch. f. Tierheilkunde, 43, S. 239-261.

161. TURNER, C.W., A.H. FRANK, C.H. LOMAS and G.W. HIBLER.
"A study of the estrus producing hormone in the urine of cattle during pregnancy."
1930: Agric. Exp. Sta. Res. Bull., 150, Missouri.

162./

162.*VELU, H.
1931: Note to Soc. Path. Exotique December.
Quoted from (8).

163.* VERNE, J. and P. ISIDOR.
"Sur la presence de cellules pigmentaires rameuses dans la muqueuse uterine."
1928: Ann. Anat. Path. et Med. Chir., 5, pp. 785-787.

164. VILHJELMSSON.
Private communication.

165. WALLACE, R.
"Farm livestock of Great Britain."
1923: Edinburgh: Oliver and Boyd. 5th Ed.

166. WEBER, E.
"Untersuchungen über die Brunst des Rindes."
1911: Arch. f. Wiss u. Prakt. Tierheilkunde, 37, S, 382-406, 443-454.

167. WIESNER, B.P.
Personal communication.

168. WILSON, K.M.
"Histological changes in the vaginal mucosa of the sow in relation to the oestrous cycle."
1926: Amer. Jour. Anat., 37, pp. 417-431.

169. ZIETSCHMANN, O.
"Lehrbuch der Entwicklungsgeschichte der Haustiere."
1924: Berlin: Schoetz.

170. ZUCKERMAN, S.
"The social life of monkeys and apes."
1932: London: Keagan Paul.

171: ZUPP, B.A.
"Studies on the oestrual flow of the pig."
1924: Anat. Rec., 27, p. 244, (Abst.)

---

* References marked thus were taken from reviews or abstracts and have not been confirmed from the original papers.

A P P E N D I X.

APPENDIX TABLE I.

Stages of Reproductive Cycle at which Experimental and other Ewes were slaughtered.

Experimental Ewes.									
E.E. 817 :	Pro-oestrum;	about	6	hours	before	commencement	of	heat.	
E.E. 828 :	Oestrum;		8	"	after	"	"	"	
E.E. 826 :	Oestrum;		24	"	"	"	"	"	
E.E. 805 :	Metoestrum;	2 days	16	"	"	"	"	"	;
			16	"	"	end of heat.			
E.E. 631 :	Metoestrum;	4 "	9	"	"	commencement	of	heat;	
		2 "	16	"	"	end of heat.			
E.E. 706 :	Metoestrum;	5 "	-	"	"	commencement	of	heat;	
		4 "	-	"	"	end of heat.			
E.E. 716 :	Dioestrum;	11 "	-	"	"	commencement	of	heat.	
Non-Experimental Ewes.									
Lamb 75 :	Anoestrum;	15th September.	Ovaries	inactive.					
Lamb 76 :	Anoestrum;	30th September.	"	"					
I.E. 490 :	Anoestrum;	7th October.	"	"					
I.E. 555 :	Anoestrum;	30th September.	"	active.	("Metoestrum" type)				
I.E. 79 :	Anoestrum;	30th September.	"	"	("Oestrum" type)				

APPENDIX TABLE I.

Stages of Reproductive Cycle at which Experimental and other Ewes were slaughtered.

Experimental Ewes.

E.E. 817 :	Pro-oestrum;	about	6	hours	before	commencement	of	heat.
E.E. 828 :	Oestrum;		8	"	after	"	"	"
E.E. 826 :	Oestrum;		24	"	"	"	"	"
E.E. 805 :	Metoestrum;	2 days	16	"	"	"	"	" ;
			16	"	"	end of heat.		
E.E. 631 :	Metoestrum;	4 "	9	"	"	commencement	of	heat;
		2 "	16	"	"	end of heat.		
E.E. 706 :	Metoestrum;	5 "	-	"	"	commencement	of	heat;
		4 "	-	"	"	end of heat.		
E.E. 716 :	Dioestrum;	11 "	-	"	"	commencement	of	heat.

Non-Experimental Ewes.

Lamb 75 :	Anoestrum;	15th September.	Ovaries inactive.	
Lamb 76 :	Anoestrum;	30th September.	" "	
I.E. 490 :	Anoestrum;	7th October.	" "	
I.E. 555 :	Anoestrum;	30th September.	" active.	("Metoestrum" type)
I.E. 79 :	Anoestrum;	30th September.	" "	("Oestrum" type)
I.E. 262 :	Anoestrum;	7th October.	" "	("Metoestrum" type)

OVER

APPENDIX TABLE I.

(Continued)

I.E. 258 :	Anoestrum;	10th October.	Ovaries active.	("Metoestrum" type)
I.E. 63 :	Anoestrum;	10th October.	" "	("Metoestrum" type)
I.E. 473 :	Oestrum;	1 hour after commencement of heat.		
I.E. 754 :	Oestrum;	6 hours "	" " "	
I.E. 736 :	Oestrum;	18 " "	" " "	
I.E. 77 :	Metoestrum;	7½ days "	" " "	
I.E. 85 :	Metoestrum;	8½ " "	" " "	
I.E. 212 :	Pregnant;	8 " "	service.	
I.E. 220 :	Pregnant;	12 " "	"	
I.E. 732 :	Pregnant;	14 " "	"	
I.E. 399 :	Pregnant;	18 " "	"	
I.E. 33 :	Pregnant;	20 " "	"	
I.E. 81 :	Pregnant;	22 " "	"	
I.E. 65 :	Pregnant;	27 " "	"	
I.E. 82 :	Pregnant;	36 " "	"	
I.E. 41 :	Parturient;	7 " "	parturition.	

APPENDIX TABLE II.

Duration of Dioestrous Cycle.

Authority.	Breed of Sheep.	Country	No. of Cycles.	Range of Variation. (Days)	Mode (Days)	Mean (Days)
Quinlan & Maré. (130)	Merino	South Africa.	481	6 - 68	17	-
Darlow & Hawkins. (47)	Merino	North America.	168	13 - 21	17	17.3
McKenzie & Phillips. (112)	Hampshires, Shropshires, Southdowns.	North America.	116	8 - 49	15 - 16	16.6
Casida & McKenzie. (37)	Hampshires, Cross-breds.	North America.	34	7 - 34½	16¼	-
Grant.	Mixed Scottish Breeds.	Scotland - Lowlands.	63	15 - 18½	16½	16.4
Marshall. (101)	Scottish Lowland Breeds.	Scotland - Lowlands.	-	13 - 18	-	-
	Blackfaced.	Scotland - Lowlands.	-	15 - 16	-	-
	Blackfaced.	Scotland - Highlands.	-	-	-	-

OVER.

APPENDIX TABLE II.

(Continued)

Küpfer. (86)	Swiss, Steirer & Bergamasker.	Central Europe.	-	-	21	-
	Persian-Woolled.	South Africa.	-	-	18	-
Beard. (13)		-	-	15 - 16	-	-
Coffey. (38)		North America.	-	12 - 28	16	-
Alongi. (4)		Sicily,	-	-	15	-
		Italy (Umbria)	-	-	20	-
Allen, McKenzie, et al. (2)		North America.	-	-	-	16.5
Cole & Miller. (40)		North America.	-	16 - 23	-	17
Sanctis. (141)		Italy (Umbria)	-	-	20 - 30	-
Schmaltz. (144)		Germany	-	-	-	21

APPENDIX TABLE III.

Duration in Days of Dioestrous Cycle in 17 Experimental Ewes		Average.
701: Shetland	15 16 $15\frac{3}{4}$ 16 $16\frac{3}{4}$ (Inseminated)	16
705: Shetland	$16\frac{1}{2}$ 17 $17\frac{1}{4}$ $15\frac{3}{4}$ $18\frac{1}{2}$	17
714: Shetland	15 16 $16\frac{3}{4}$ $16\frac{1}{2}$ $16\frac{1}{2}$ $17\frac{1}{4}$ $17\frac{1}{2}$ 17 $17\frac{1}{2}$	$16\frac{1}{2}$
726: Blackface	$16\frac{3}{4}$ (Inseminated: Foetus removed) $15\frac{1}{2}$ $16\frac{1}{2}$ $16\frac{1}{4}$ $17\frac{1}{4}$	$16\frac{3}{4}$
631: Blackface	$16\frac{3}{4}$ (Killed)	$16\frac{3}{4}$
828: Cheviot	$16\frac{1}{2}$ $16\frac{3}{4}$ (Killed)	$16\frac{1}{2}$
805: Cheviot	$16\frac{1}{4}$ $16\frac{1}{2}$ (Killed)	$16\frac{1}{4}$
816: Cheviot	$16\frac{3}{4}$ $16\frac{3}{4}$ $16\frac{1}{4}$ $16\frac{3}{4}$ 22* 12 $17\frac{1}{4}$ $17\frac{1}{2}$ $16\frac{3}{4}$ $17\frac{1}{4}$ $18\frac{1}{2}$	17
817: Cheviot	$16\frac{1}{2}$ $16\frac{1}{2}$ (Killed)	$16\frac{1}{2}$
826: Cheviot	$16\frac{1}{2}$ $16\frac{1}{2}$ (killed)	$16\frac{1}{2}$
710: Shetland x Cheviot	$16\frac{1}{4}$ (Inseminated: Foetus removed) $14\frac{1}{4}$ 11 6	$16\frac{1}{4}$
704: Cheviot x Shetland	($15\frac{1}{2}$) $16\frac{1}{2}$ $15\frac{3}{4}$ $16\frac{3}{4}$ $17\frac{1}{4}$ (Corpus luteum removed) $19\frac{3}{4}$ (Inseminated)	$16\frac{1}{4}$

OVER.

APPENDIX TABLE III.

(Continued)

773: Cheviot x Shetland	15¾ 17 15¾ 17½ 17 18½	17
706: Cheviot x Blackface	15¼ (Killed)	15¼
716: Cheviot x Lincoln	15½ 15¼ (Killed)	15½
333: Lincoln x Blackface	16½ 16½ 16 16 16¼ (17)	16¼
751: Gritstone x Blackface	15½ 16¼ 17 17¾	16½
Number of Cycles	17 13 8 8 6 3 2 2 2 1 1	
Average length	16 16½ 16¼ 16½ 17 17½ 16¼ 17¼ 17 17¼ 18½	

The average length of cycle (65 normal cycles from unoperated ewes) is 16.4 days.

* The "heat" reaction seems to have been delayed in some way as the oestrous smear returned after the normal interval of 16½ days.

APPENDIX TABLE IV.

Duration in Hours of Oestrous Periods in 17 Experimental Ewes		Average.
701: Shetland	76 57 47 51 77 72 (Inseminated)	63
705: Shetland	33 44 54 26 24 14	32
714: Shetland	15 24 25 24 40 60 56 54 70 84	45
726: Blackface	14 29 (Inseminated: Foetus removed) 23 30 20 29 27	21
631: Blackface	22 42 (Killed)	32
828: Cheviot	20 40 (Killed)	30
805: Cheviot	25 48 60 (Killed)	44
816: Cheviot	33 31 28 42 48 48 48 36 36 36 36 27	37
817: Cheviot	30 35 (Killed)	32
826: Cheviot	30 24 24+ (Killed)	27
710: Shetland x Cheviot	12 26 (Inseminated: Foetus removed) 23 30 20 29 27	19
704: Cheviot x Shetland	46 60 36 40 26 (corpus luteum removed) 64 (Inseminated)	42
773: Cheviot x Shetland	11 30 30 26 12 28 3	20

OVER.

APPENDIX TABLE IV.

(Continued)

706: Cheviot x Blackface	32	60	(Killed)									46
716: Cheviot x Lincoln	18	36	66	(Killed)								40
333: Lincoln x Blackface	24	23	24	18	24	22						23
751: Gritstone x Blackface	44	40	32	28	18							32
Number of Periods	17	17	10	8	8	6	3	2	2	2	1	1
Average Length	29	38	40	32	34	41	36	45	53	60	36	27

Average Duration of Heat Period (77 periods) 36.2 hours.

APPENDIX TABLE V.

Duration of Oestrum.

Authority.	Breed of Sheep.	Country.	No. of Periods.	Range of Variation. (Hours)	Mode. (Hours)	Mean. (Hours)
McKenzie & Phillips. (112)	Hampshire, Shropshire & Southdown ewes.	North America.	247	5 - 50	25	26.8
	Hampshire & Shropshire lambs.	North America.	-	8 - 41	-	19.5
Quinlan & Maré. (130)	Merino.	South Africa.	77	24 - 96	36 - 48	40
Cole & Miller (41)	Rambouillet.	North America	Abt. 100	24 - 72	30 - 40	-
Grant.	Mixed Scot-:tish Breeds.	Scotland - Lowlands.	77	3 - 84	22 - 34	36
Allen, et al. (2)		North America.	-	-	-	27
Wallace. (165)		Scotland.	-	-	-	24
Coffey.(38)		N.America.	-	-	-	48
Küpfer.(86)	Persian-Woolled.	South Africa.	-	-	48	-
Alongi.(4)		Sicily	-	-	24 - 48	-
		Italy (Umbria)	-	-	48	-

APPENDIX TABLE VI.

Mean Duration of Oestrous Period in Different Breeds.

Authority	Breed	Range of Variation	Mean Duration
McKenzie & Phillips. (112)	Hampshire	-	30.7
	Shropshire	-	26.3
	Southdown	-	24
Quinlan & Mare. (130)	Merino	24-96	40
Grant.	Cheviot	20-60	35

APPENDIX TABLE VII.

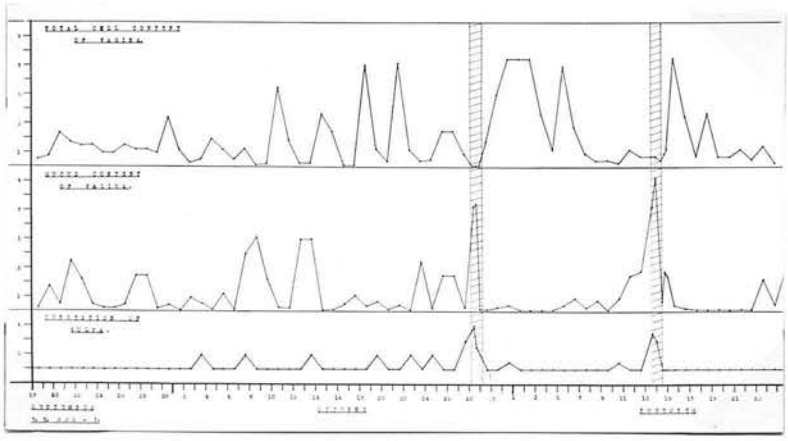
Duration in Days of Interoestrous Period in 17 Experimental Ewes.

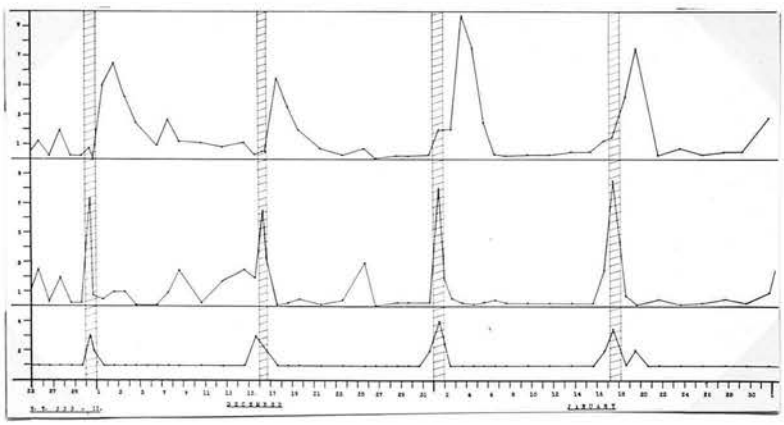
701:	11¾	13½	13¾	14	13½						
705:	15	15¼	15	14¾	17½						
714:	14¼	15	15¾	15½	14¾	14¾	15¼	14¾	14½		
726:	16¼										
631:	15¾										
828:	15¾	15									
805:	15¼	14½									
816:	15¼	15½	15	15	-	-	15¼	16	15¼	15¾	17
817:	15¼	15									
826:	15¼	15½									
710:	15¾										
704:	14½	13¼	15¼	15½							
773:	15¼	15¾	14½	16½	16½	17¼					
706:	14										
716:	14¾	13¾									
333:	15½	15½	15	15¼	15¼	16					
751:	13¾	14½	15¾	16½							

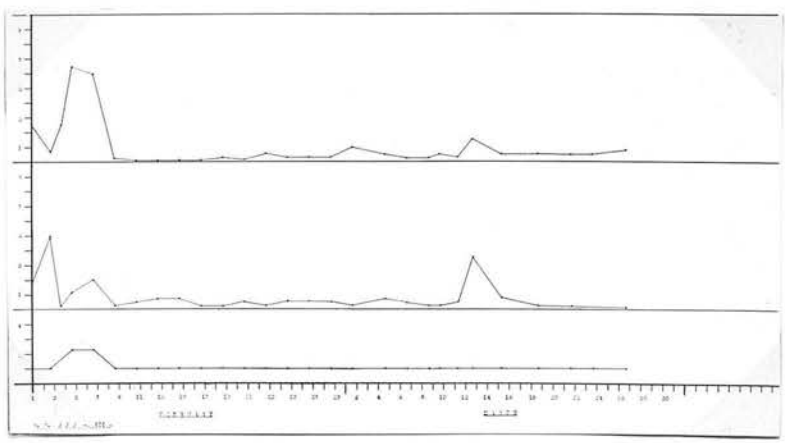
GRAPHICAL RECORDS OF VAGINAL CHANGES IN EXPERIMENTAL EWES.

The cross-hatched areas in these graphs represent the oestrous periods.

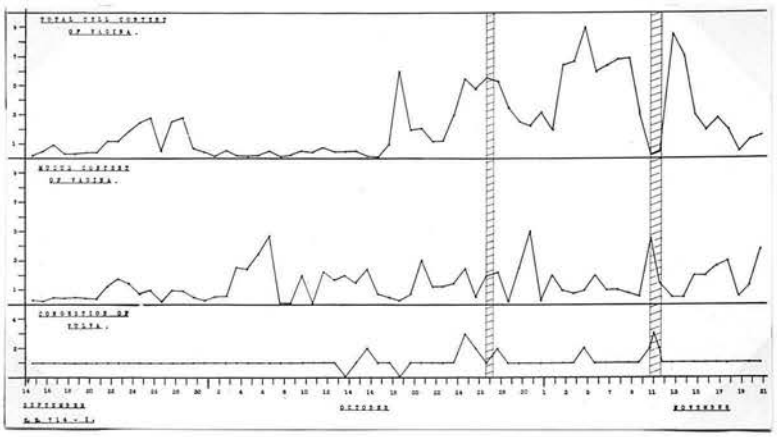
Experimental Ewe 333 : Lincoln ♂ x Blackfaced ♀.

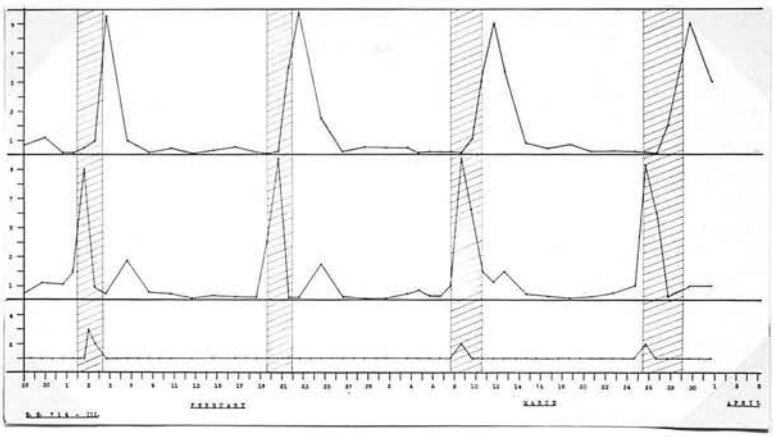


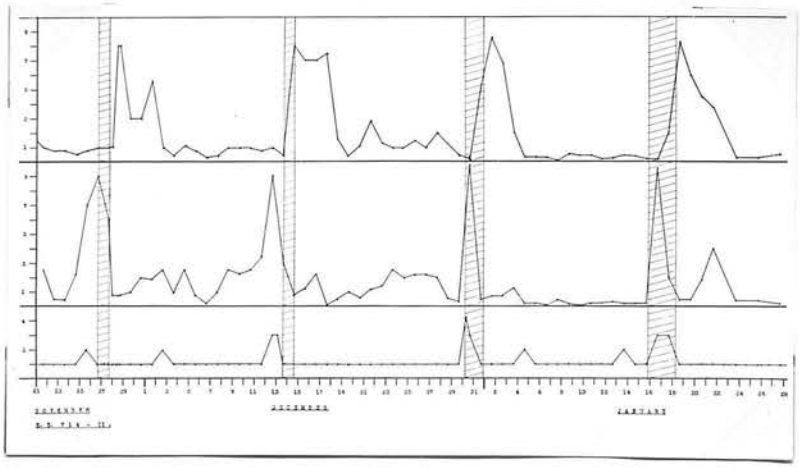




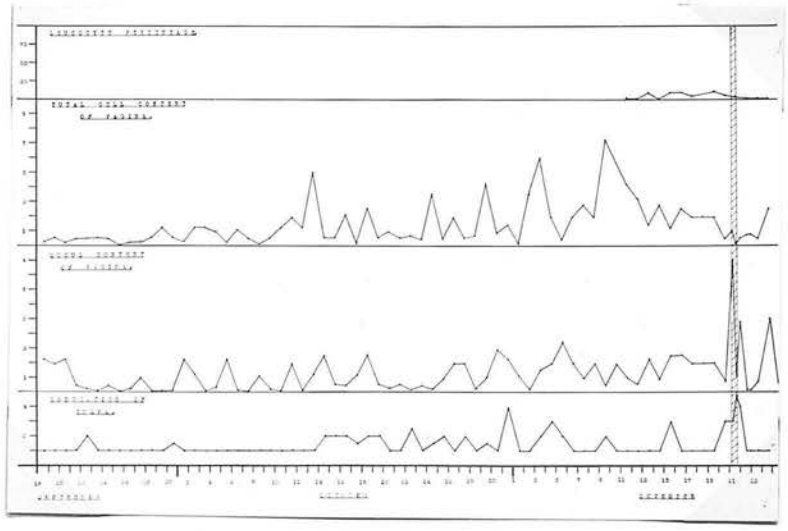
Experimental Ewe 714 : Shetland.

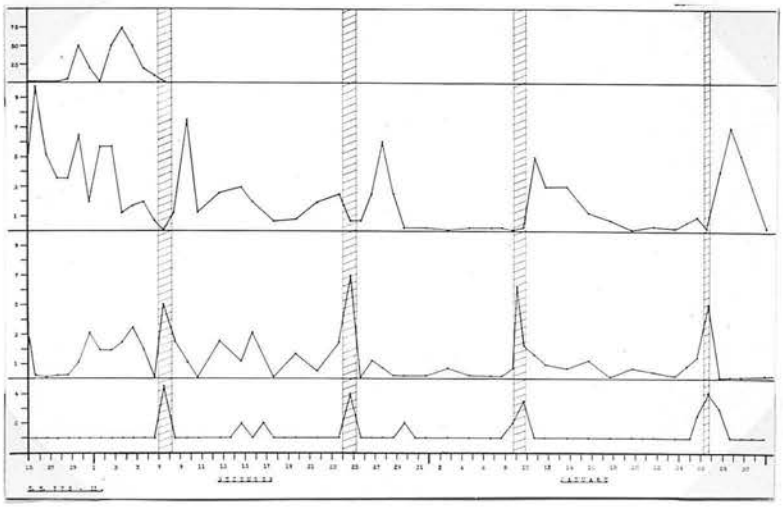


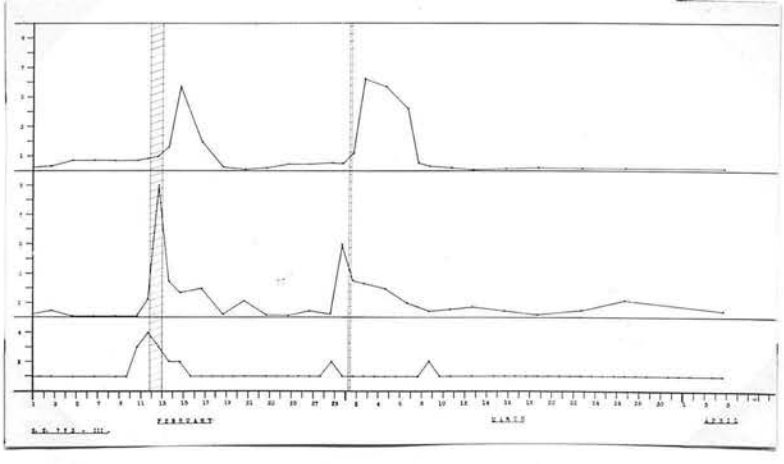




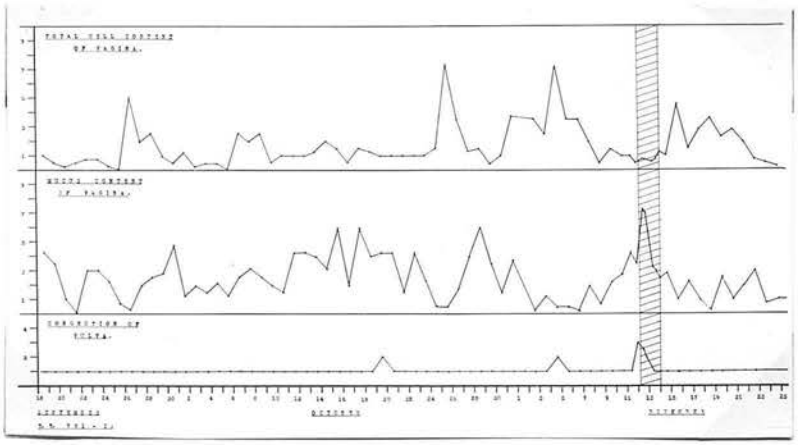
Experimental Ewe 773 : Cheviot♂ x Shetland ♀.

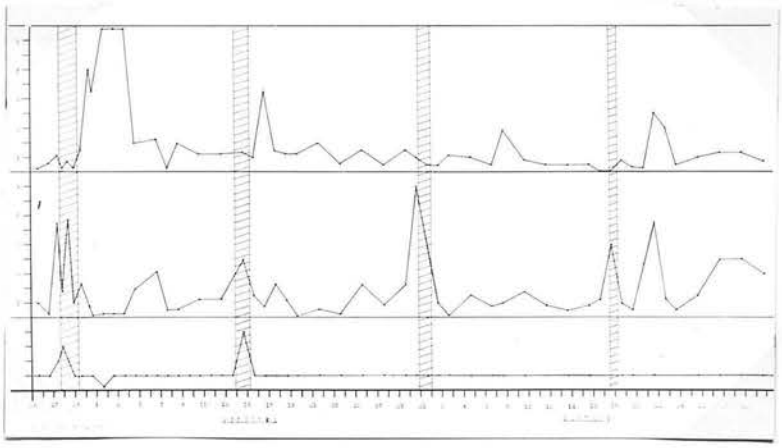


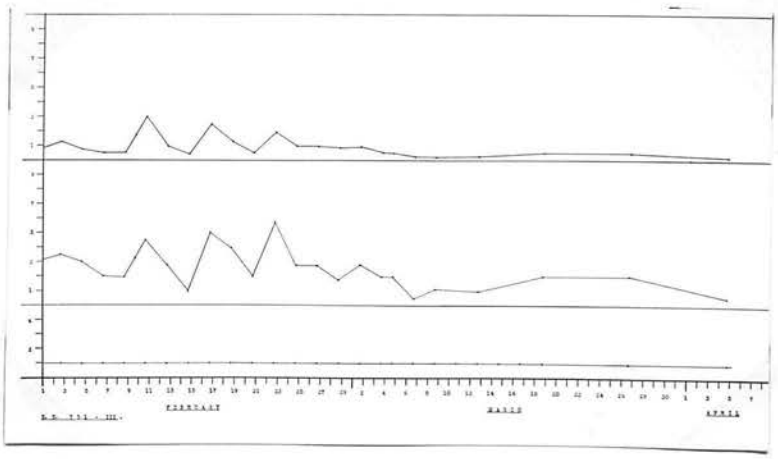




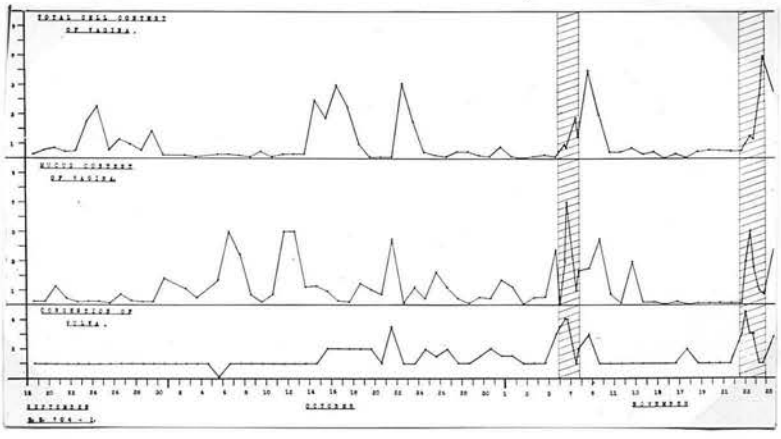
Experimental Ewe 751 : Gritstone ♂ x Blackfaced ♀.

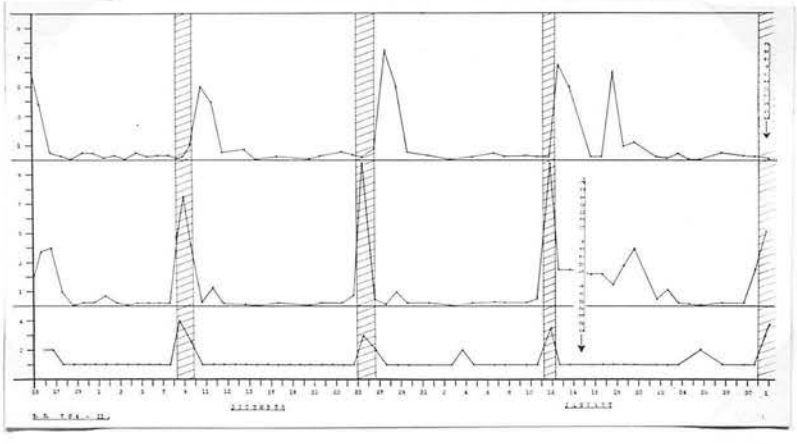


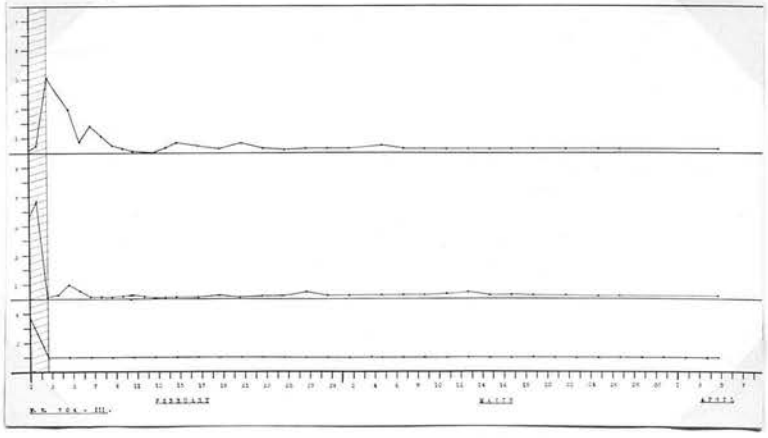




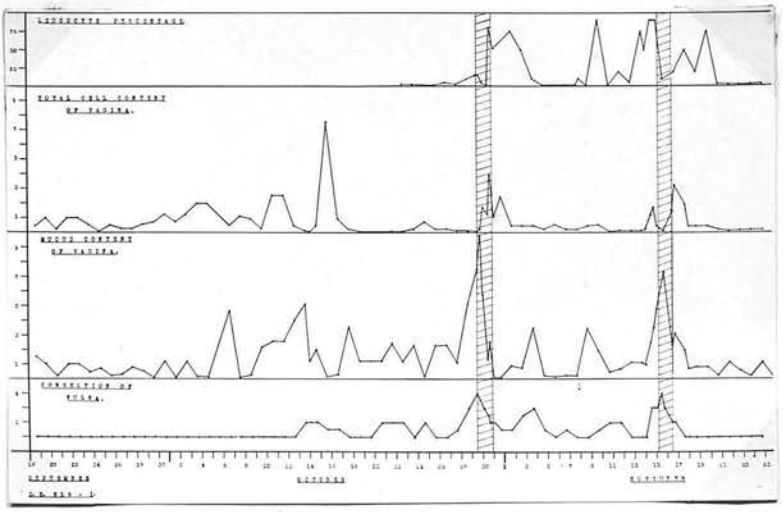
Experimental Ewe 704 : Cheviot ♂ x Shetland ♀.

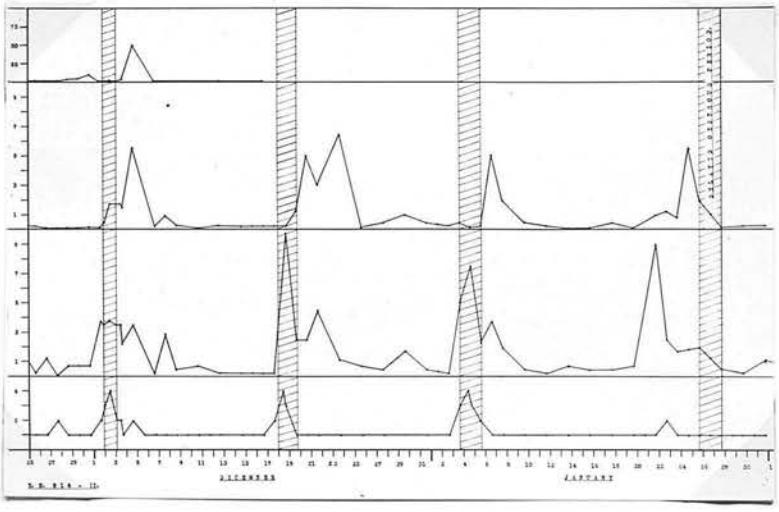


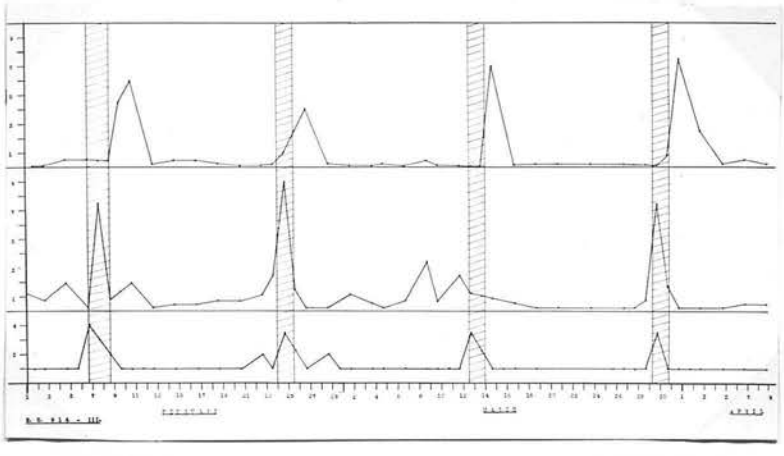


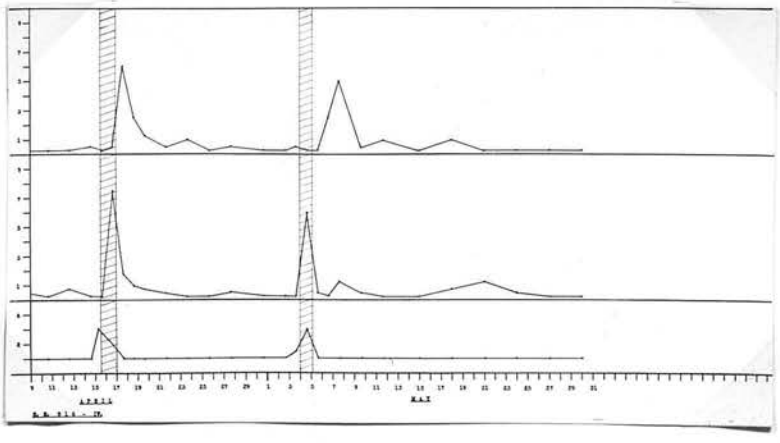


Experimental Ewe 816 : Cheviot (North Country).

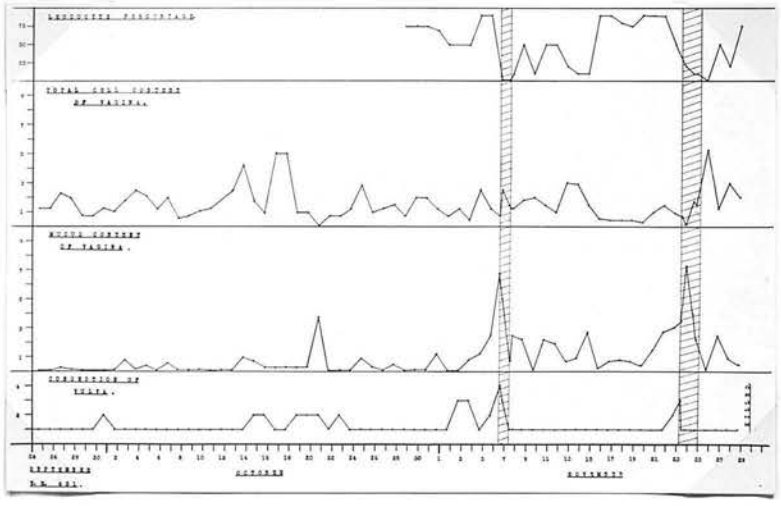




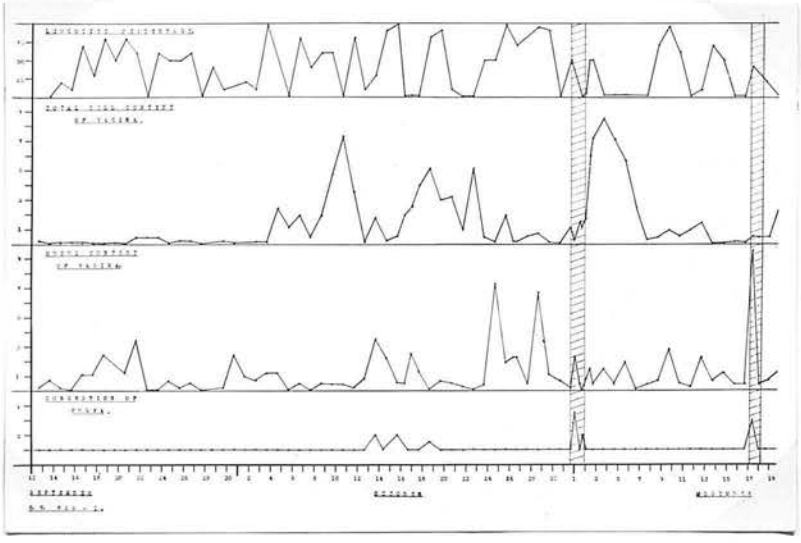


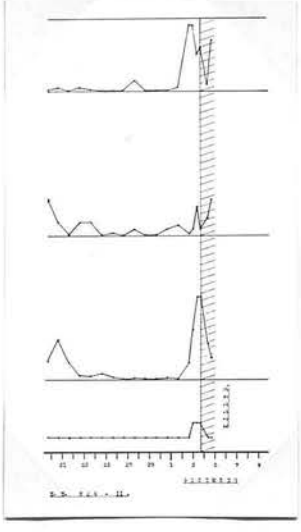


Experimental Ewe 631 : Blackfaced.

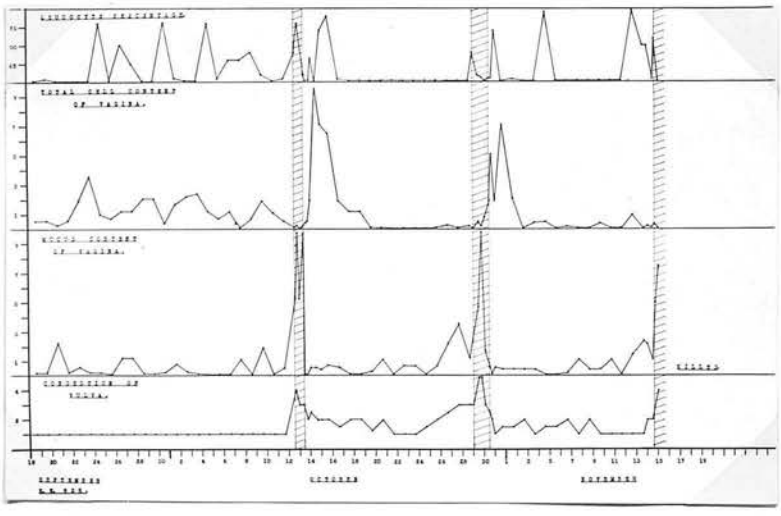


Experimental Ewe 826 : Cheviot (North Country).

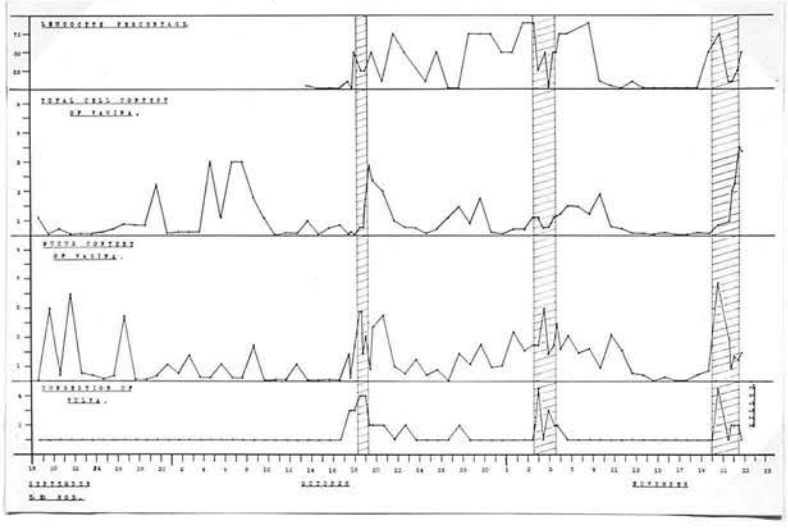




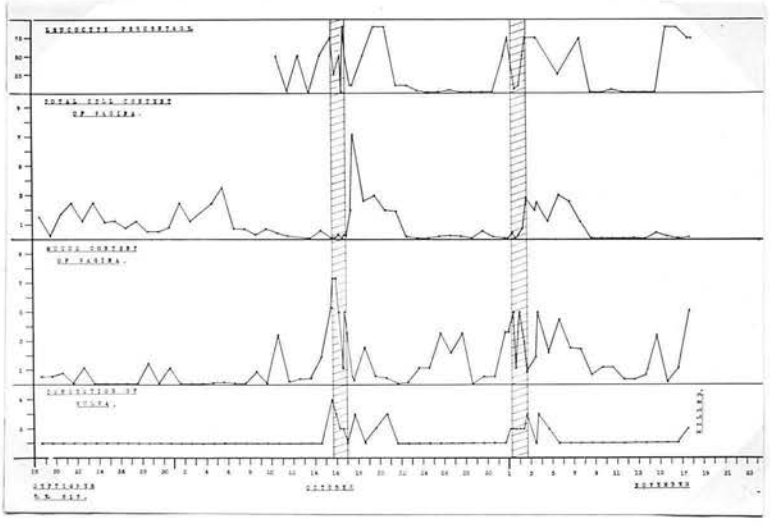
Experimental Ewe 828 : Cheviot (North Country).



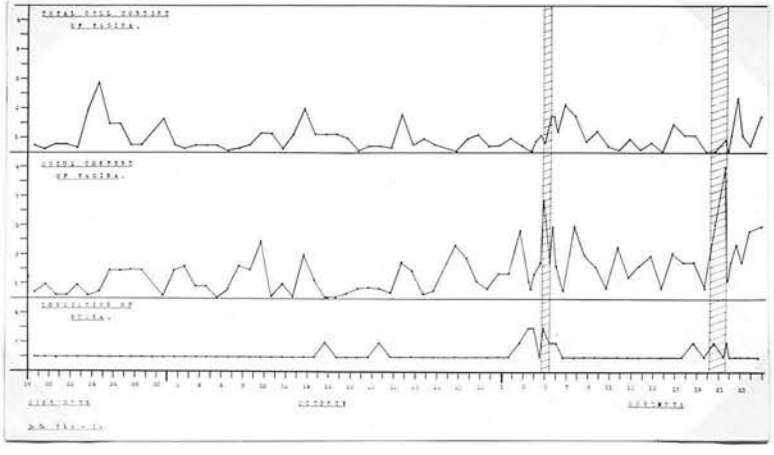
Experimental Ewe 805 : Cheviot (North Country).

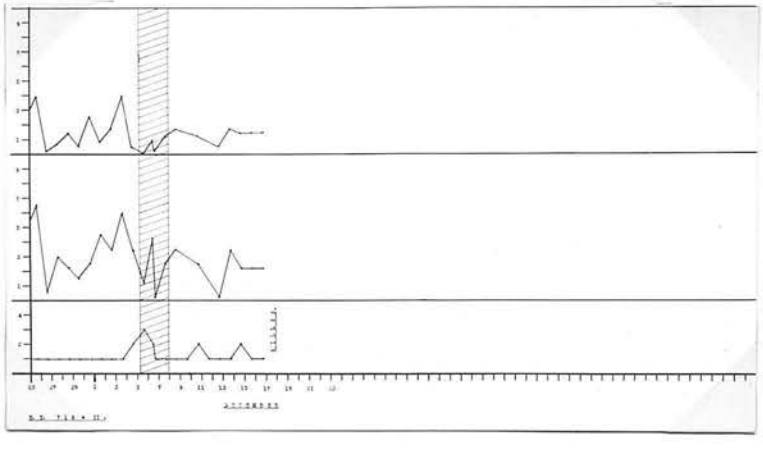


Experimental Ewe 817 : Cheviot (North Country).

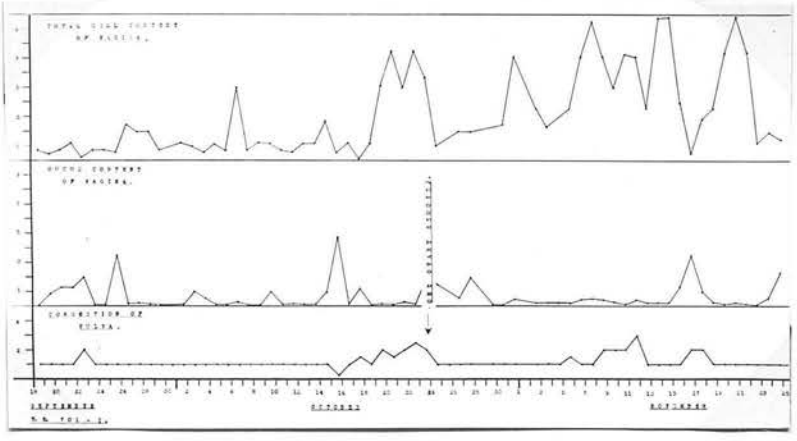


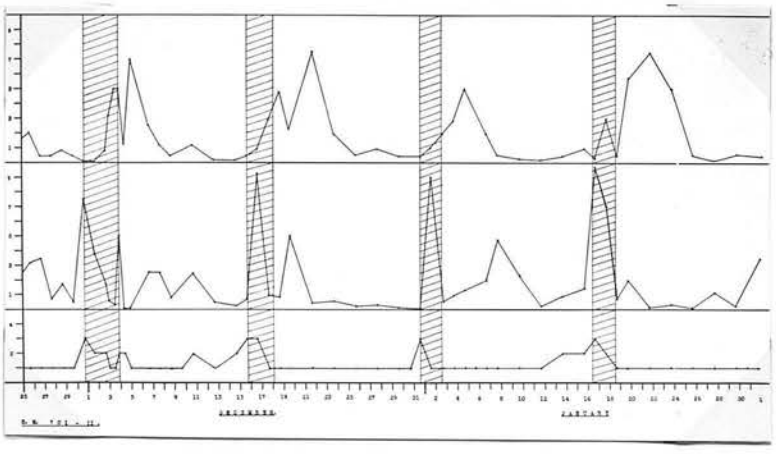
Experimental Ewe 716 : Cheviot ♂ x Lincoln ♀.

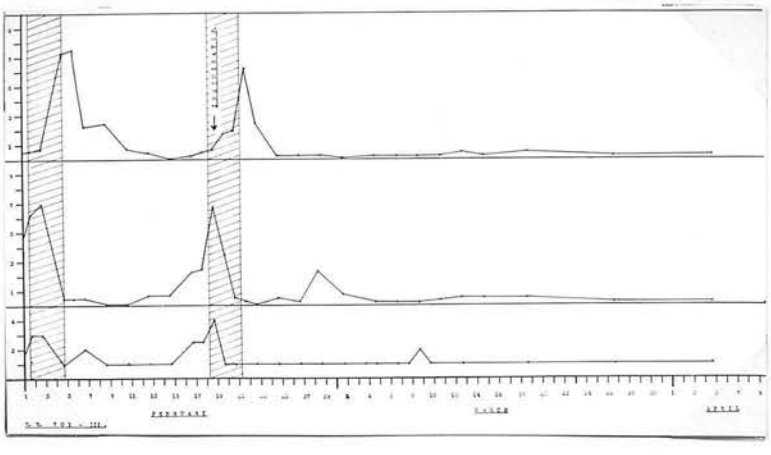




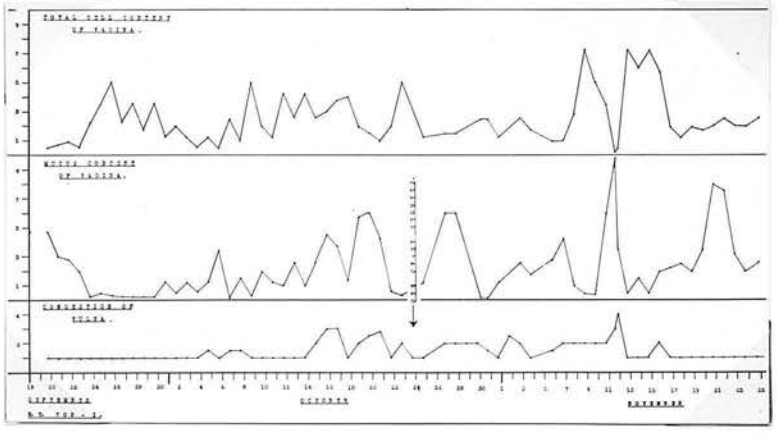
rimental Ewe 701 : Shetland (Unilaterally Ovariotomised).

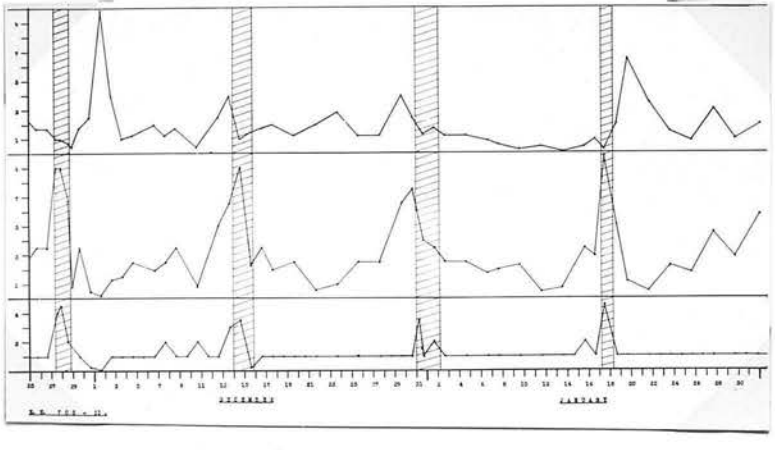


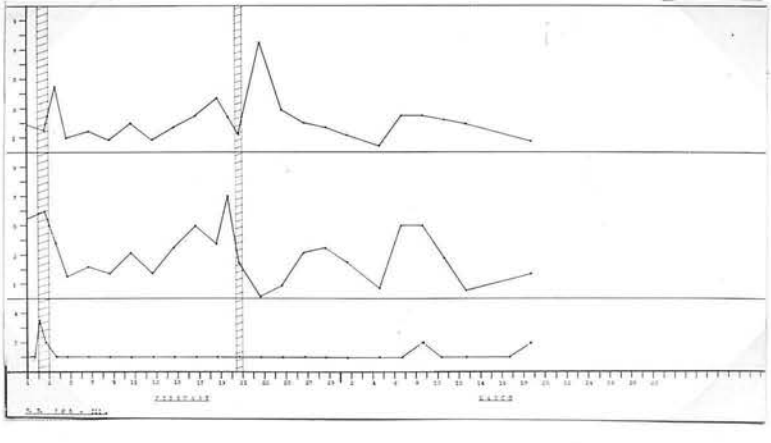


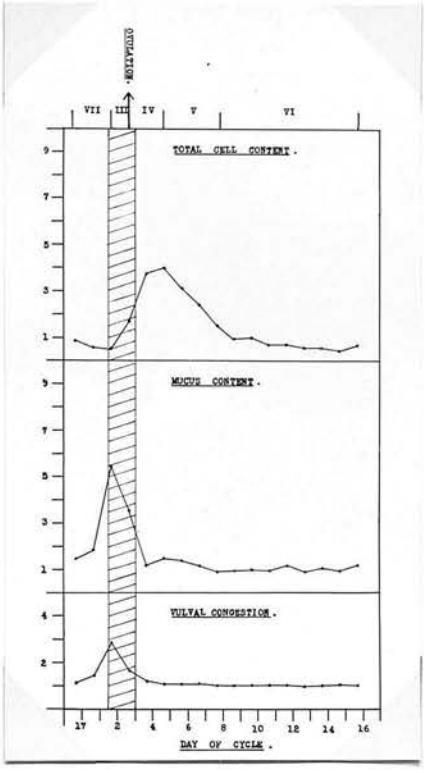


erimental Ewe 705 : Shetland (Unilaterally Ovariotomised).









Mean Graphical Record of vaginal smear changes for all experimental ewes (see text p. 216). The approximate time of ovulation and the phases of the oestrous cycle represented by the five classes of slaughter house material are shown at the top of the figure.